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The American Phytopathological Society

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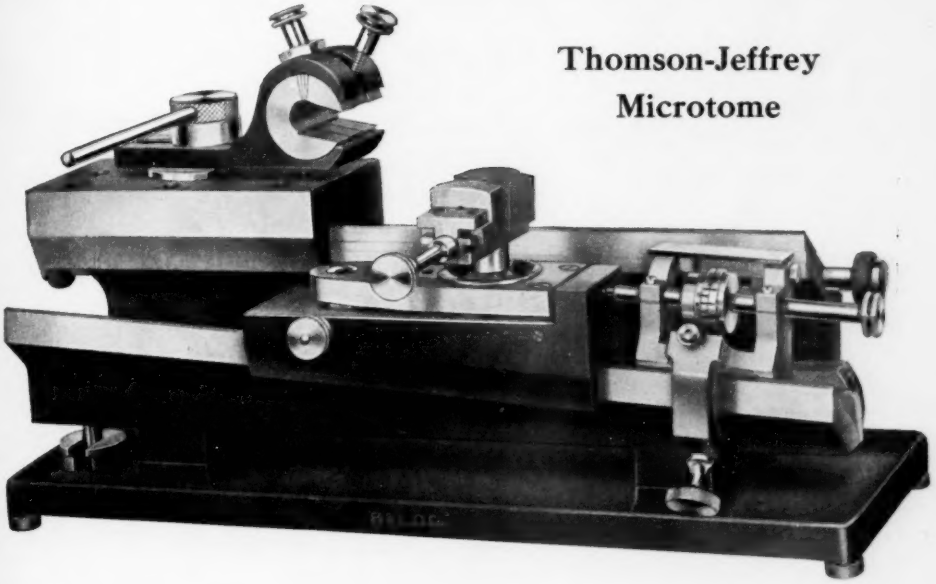

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THE THERMAL DEATH RATE OF TOBACCO-MOSAIC VIRUS

W. C. PRICE[1]

(Accepted for publication July 29, 1933)[2]

INTRODUCTION

The relation between temperature and inactivation of plant viruses is useful in allowing separation of groups of viruses. Resistance of tobacco-mosaic virus to high temperature has been studied by a number of investigators. For the most part, these studies were limited to determinations of the maximum temperature to which this virus might be heated without losing its infectivity. Very little is known about the rate of inactivation of tobacco-mosaic virus at any given temperature or of the effect of different temperatures for a given length of time. The lack of a suitable and adequate method for measuring virus concentration has made it difficult and tedious to study such factors as the gradual diminution in virus concentration due to heating. Because of the recent development of the local lesion method by Holmes (5) and a modification of this method (13), it has been possible to make a quantitative study of the effect of heat on the virus of tobacco mosaic. The purpose of this paper is to present the results of this study.

REVIEW OF LITERATURE

Mayer (10) reported that the infectivity of sap of diseased plants was not perceptibly changed by continued heating at 60° C., that it was weakened by heating to 65°–70° C., and that it was completely destroyed by heating for several hours at 80° C. Iwanowsky (6) found that the infectious property of juice from mosaic-diseased plants was completely lost when heated to temperatures near the boiling-point. Beijerinck (2) stated that tobacco-mosaic virus was inactivated by boiling or when kept for a short time at 90° C. Raciborsky (14) reported that sap from diseased plants did not lose its infectivity when heated for five minutes at 62° C. or for one minute at 100° C., but that its infectivity was completely destroyed when heated for fifteen minutes at 100° C. Koning (9) con-

[1] This work was begun while the writer was employed by the Boyce Thompson Institute for Plant Research, Yonkers, New York.

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cluded that virus diluted 1:10 with water and heated in closed tubes remained infectious when the heating process was carried on for ten minutes at 80° C., five minutes at 90° C., or five minutes at 100° C., although it was considerably weakened by heating for five minutes at 100° C. Woods (16) stated that boiling did not completely inactivate tobacco-mosaic virus in expressed juice. Allard (1) found that the thermal death point of tobacco-mosaic virus in freshly expressed juice was in the neighborhood of 90° C. for ten minutes and that the virus was quickly destroyed at temperatures above this point. He also showed that virus in dried material required much more heat for inactivation than that in solution.

Mulvania (12) found that tobacco-mosaic virus was completely inactivated when heated for ten minutes at 90° C. and was considerably weakened when heated for the same length of time at temperatures slightly below this. He observed, on the other hand, that a similar sample of virus was not completely inactivated when heated for as long as three days at 80° C. Mulvania did not observe any correlation between the water content of a virus sample and its thermal death point. McKinney (11) found that the thermal death point of virus in freshly expressed juice of mosaic-diseased plants was between 88° C. and 90° C. for ten minutes and that the thermal death point of a similar virus sample in a water dilution of 1:100 was between 82° and 84° C. for ten minutes. He concluded that the thermal inactivation of tobacco-mosaic virus is affected by the concentration of virus in the inoculum and also by the plant constituents in which the virus is contained. Brewer *et al.* (4) found that tobacco-mosaic virus (Johnson's tobacco virus 1) in freshly expressed juice of diseased tomato was completely inactivated when held at 88°–90° C. for ten minutes, or at 82°–84° C. for twenty-five minutes.

Recently, Johnson and Grant (8) have shown that tobacco-mosaic virus is not always completely inactivated when heated for ten minutes at 90° C. but that the thermal death point is between 90° and 95° C. for ten minutes. They also studied the influence of host plant on thermal inactivation of tobacco-mosaic virus and found that the thermal death point varied as much as 5° C., depending on the host species from which the virus was obtained. Whether this variation is the result of a lower concentration of virus in some hosts, or of the effect of the host juices, was not ascertained.

MATERIALS AND METHODS

The studies reported in this paper were conducted with ordinary tobacco-virus, classified by Johnson (7) as tobacco virus 1 and used by Holmes (5) and Price (13) in their studies on local lesions of tobacco mosaic. The virus samples used for heating experiments were obtained

from diseased plants of *Nicotiana tabacum* L. var. Turkish, which had been infected for 30 or more days. Juice was extracted from such plants by grinding in a meat chopper and pressing by hand through one thickness of cheese-cloth.

Method of heating virus samples. In the studies where it was necessary to maintain a uniform temperature for only a short period of time (periods up to two hours), the virus samples were immersed in an electrically heated and stirred water bath, held within 0.2° C. of the desired temperature. At the end of the heat treatment, the virus samples were plunged into cold water. In such tests the virus samples were placed in containers prepared from glass tubing of 3–4 mm. bore and in 25–30 mm. lengths. At the time of testing a virus sample, one end of a tube was drawn to a fine point in a flame. Approximately two cc. (one cc. for the one-minute tests) of the sample to be tested were drawn into the tube, one end of which was then carefully sealed by heat. This treatment did not measurably decrease the infectivity of the virus sample, as was shown by tests conducted with samples immediately after preparation.

Tests were made to determine the length of time required for a virus sample to reach the temperature of the water bath in which it was placed. These tests were made by the use of a thermocouple attached to a galvanometer. The results of the tests showed that from 40 to 45 seconds were required for the temperature of the solution in the tube to reach the temperature of the bath. In 30 to 35 seconds after immersion there was a difference of one degree in temperature of the two solutions; in 25 seconds there was a difference of four degrees in temperature. The tests were made in a water bath held at temperatures of 100° C., 90° C., and 80° C., respectively. In the heating experiments shown below, no allowance was made for the time required for the temperature of the virus solution to reach the temperature of the bath. The time periods shown in the table represent the actual time the tubes were immersed. Thus, a virus solution that was kept for one minute in a bath held at 95° C. was actually maintained at 95° C. for only 15 to 20 seconds, from 95° to 94° C. for ten seconds, and from 94° to 91° C. for ten seconds.

In experiments in which it was necessary to maintain a uniform temperature over a period of time longer than two hours, a slightly different procedure was followed. Approximately three cc. of a virus sample were placed in a tightly stoppered 1 x 10 cm. test tube. Such tubes were kept in electrically heated ovens for the duration of the test. The temperature was taken at the level of the tubes in the ovens. The oven temperature, as a rule, did not vary more than $\pm$ 1° C., but occasionally a variation of as much as 2° C. was observed. In the later experiments the tubes of virus were immersed in a paraffin bath kept inside the ovens.

Method for measuring virus concentration. The method employed by previous workers for testing for presence of virus in samples of heated juice depended upon the appearance of the systemic disease in plants of *Nicotiana tabacum* inoculated with such samples. Usually five or ten plants were used for each sample tested and the results were given in percentage of infection. The appearance of the systemic disease in this host indicates the presence of virus in the inoculum but gives little indication of the relative concentration of virus unless a large number of plants are used. The local lesion method (5, 13) for measuring virus concentration not only differentiates between presence and absence of virus but also allows a reasonably accurate estimation of virus concentration. The method was employed in the studies reported in this paper. Lesions made their appearance in about 36 hours and were usually counted in five or more days after inoculation. The test plants were grown in a greenhouse in which the temperature was not allowed to fall below 68° F.

Some of the experiments reported in this paper were conducted with *Nicotiana glutinosa* L. as the test plant and the remainder with *Phaseolus vulgaris* L. var. Early Golden Cluster as the test plant. Several of the experiments with *N. glutinosa* were duplicated with *P. vulgaris,* thus allowing a comparison to be made between the two test plants. It is of interest to note that there was a close agreement between the results obtained with these two hosts.

In cases in which *Nicotiana glutinosa* was used as the test plant, five leaves of this host were inoculated with the sample tested. Plants used in these experiments were selected for uniformity of leaves at the time of making the test. In those cases in which *Phaseolus vulgaris* was used as the test plant, each virus sample was tested by inoculation of 16 primary leaves, which were almost fully expanded.

EXPERIMENTAL

Thermal death point is usually defined as the minimum temperature that results in complete inactivation after exposure for a definite period of time under specific conditions. A ten-minute period is usually taken as a standard. Similarly, thermal death time may be defined as the length of time required for complete inactivation of a virus sample when exposed to a definite temperature under specific conditions. The writer determined the thermal death point of tobacco-mosaic virus for a ten-minute exposure and for a one-minute exposure and the thermal death time for temperatures of 68°, 75°, 80°, 85°, and 90° C. The details of these experiments are given below.

THERMAL DEATH POINT: TEN-MINUTE EXPOSURE

Seven different experiments were conducted in order to determine the thermal death point (ten-minute exposure) of tobacco-mosaic virus in freshly expressed juice of diseased Turkish tobacco plants. These experiments were conducted at different times of the year and included seven different virus samples. Table 1 shows the results of four experiments in which *Nicotiana glutinosa* was used as a test plant. The results of three experiments in which Early Golden Cluster beans were used as test plants are given in table 2. These results indicate that there is a slight variation in thermal death point of different samples of virus, the minimum thermal death point observed being 91° C., the maximum 93° C. On the other hand, it is possible that this seeming inconsistency resulted from variation in test plants and the failure to detect minute quantities of virus in some instances rather than from any variation in virus samples. It is concluded from the results of these experiments that the thermal death point of tobacco-mosaic virus in freshly expressed juice of mosaic-diseased tobacco plants is 93° C. for ten minutes.

The results given in tables 1 and 2 also show that partial inactivation of virus occurs at temperatures well below the thermal death point and that this inactivation increases with rise in temperature. In all cases observed, a temperature of 87° C. (six degrees below the thermal death point) caused a significant decrease in virus concentration.

Effect of water dilution on thermal death point: ten-minute interval. It is well known that the thermal death point of various organisms is influenced by the nature of the dispersing medium and the number of organisms in the sample. It is, therefore, of interest to know whether the thermal relations of tobacco-mosaic virus, as well as of other viruses, are influenced by such factors. It is already known that the thermal death point of tobacco-mosaic virus is appreciably lowered by dilution (11). Several experiments were undertaken in order to obtain more detailed information regarding the effect on thermal death point of dilution with water. Freshly extracted juice of mosaic-diseased plants diluted 1:20 with water was used in these experiments. Table 3 shows the results of six tests with juice diluted 1:20 with water. With three of the virus samples tested, the thermal death point was found to be 88° C. for ten minutes; with one sample, the thermal death point was 87° C.; with the remaining samples 86° C. These results show that dilution with water significantly lowers the thermal death point of tobacco-mosaic virus.

It is also of interest, in connection with these experiments, to note that, as in the case with undiluted juice, temperatures considerably below the thermal death point cause a marked decrease in virus concentration and that the degree of inactivation increases with rise in temperature.

Effect of dilution with healthy tobacco extract. Since it has been shown that the thermal death point of tobacco-mosaic virus is significantly lowered by dilution with water, it is of interest to know whether this decrease is the result of the decrease in virus concentration or of a decrease in total solids or of both. Such a question is difficult to answer until it is possible to work with a purified virus solution. Some information regarding it has been obtained, however, by using juice of healthy tobacco plants as a diluting medium. In such a dilution, the concentration of virus can be considerably lowered without any appreciable decrease in solid content of the solution. Such a solution is not strictly comparable with undiluted juice of mosaic-diseased plants, since the presence of virus in the plant no doubt alters the properties of the juices of such a plant. It does, however, allow a rough comparison of the effect of dilution of virus and dilution of solids in the dispersing medium.

Thermal death point determinations were made with juice of mosaic-diseased plants diluted 1:20 with juice of healthy tobacco plants. The failure of control plants to produce lesions when inoculated with juice from these healthy plants proved that the juice used for dilution was free of any appreciable quantity of tobacco-mosaic virus. The results of five tests with virus in freshly extracted juice of mosaic-diseased plants diluted with healthy juice are given in table 4. In two tests, the thermal death point of virus in this dilution was found to be 91° C.; in three tests, 90° C. A comparison of these results with those presented in tables 1, 2, and 3 shows that the thermal death point of virus diluted 1:20 with juice of healthy tobacco plants is two degrees lower than that of undiluted juice and three degrees higher than that of virus in juice diluted 1:20 with water. It is, therefore, concluded that the thermal death point of tobacco-mosaic virus is influenced by the concentration of virus as well as by the nature of the dispersing medium.

An interesting phenomenon is clearly shown by comparison of tables 1, 2, 3, and 4. Although the concentration of virus in the samples used for tests shown in table 4 (juice diluted 1:20 with healthy tobacco juice) was reduced 20 times, the number of lesions produced upon leaves of *Nicotiana glutinosa* by inoculation was only slightly less than that produced by inoculation with undiluted juice. The cause of this phenomenon is not known. It may be due to the presence in healthy juice of some substance that has an effect similar to that of norit or talc, as reported by Vinson and Petre (15).

Effect of filtration on thermal death point. Since the results of previous experiments showed that the thermal death point of tobacco-mosaic virus is influenced by the concentration of virus and by the concentration

TABLE 1.—*Effect of exposure of tobacco-mosaic virus to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
86	154[a]	440	1000	528	2122	530
87	104	777	657	367	1905	476
88	138	491	516	239	1384	346
89	96	209	116	435	856	214
90	69	24	10	108	211	53
91	6	0	0	18	24	6
92	1	0	0	0	1	1
93	0	0	0	1	1	1
94	0	0	0	0	0	0
95	0	0	0	0	0	0
Unheated controls	1812	2700	2650	1236	8398	2099

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

of solids in the dispersing medium, it appeared that further information regarding this phenomenon might be obtained by studying the effect of filtration. Although it is well known that tobacco-mosaic virus is infectious after passage through a Berkefeld filter candle, most workers believe that filtration reduces the concentration of virus. No one has yet determined to what degree filtration reduces virus concentration. Juice from mosaic-diseased tobacco plants was diluted 1:20 with water and immedi-

TABLE 2.—*Effect of exposure of tobacco-mosaic virus to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Total	Average
87	795[a]	2272	2936	6003	2001
88	759	3944	3312	8015	2672
89	123	1712	862	2697	899
90	50	928	512	1490	497
91	0	11	5	16	5
92	0	1	2	3	1
93	0	0	0	0	0
94	0	0	0	0	0
95	0	0	0	0	0
96	.....	0	0	0	0
Unheated controls	3435	3488	5550	12473	4158

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 3.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Test No. 6	Total	Average
77	272[a]	311	91	200	.....	.....	874	219
78	206	200	60	293	.....	.....	759	190
79	146	138	28	109	.....	.....	421	105
80	132	141	65	92	.....	.....	430	108
81	92	247	42	131	23	31	566	94
82	169	86	13	93	95	49	505	84
83	429	123	39	120	12	26	749	125
84	351	183	15	217	14	5	785	131
85	126	114	2	36	4	12	294	49
86	91	35	0	64	0	2	192	32
87	2	6	0	21	0	0	29	5
88	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0
Unheated controls	334	376	222	369	260	199	1760	293

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

ately filtered through a Berkefeld filter candle of the "N" grade. This solution was kept frozen until needed. Four thermal death-point determinations were made with juice that had been treated in this manner. The results of these tests are shown in table 5. In three of the tests, the thermal death point was found to be 84° C., in one test 85° C. The variation is obviously the result of experimental procedure, since the virus samples were identical in each case. This thermal death point is three degrees below that of unfiltered juice diluted 1:20 with water. It is, therefore, concluded that filtration lowers the thermal death point of tobacco-mosaic virus, although it is realized that freezing also may have altered the resistance of the virus to heat.

Effect of addition of gelatin on thermal death point. In order to obtain more information regarding the influence of concentration of solids on thermal relations of tobacco-mosaic virus, thermal death-point determinations were made with a virus sample to which gelatin had been added. Enough gelatin was added to each solution tested to bring the concentration of gelatin in the sample to two to four per cent by weight. Some slight dilution of the virus sample resulted from this treatment, but the dilution was not significantly large. The results of two tests with undiluted juice of mosaic-

TABLE 4.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with healthy extract) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
81	993[a]	1796	194	2100	974	6057	1211
82	378	1675	192	1250	1300	4795	959
83	441	231	98	2303	646	3719	744
84	498	1115	61	456	889	3019	604
85	395	250	70	512	342	1569	314
86	274	1367	20	184	75	1920	384
87	120	397	10	8	0	535	107
88	90	25	3	36	1	155	31
89	4	12	2	11	9	38	8
90	0	2	0	1	0	3	1
91	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0
	906	1735	270	3240	3320		
Unheated controls	1120	1928	470			19932	1533
	2134	1460	162				
	2950		237				
Healthy juice alone	0	0	0	0	0	0	0

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

diseased tobacco plants to which gelatin had been added are given in table 6. In one test with this solution, the thermal death point was found to be 93° C. for ten minutes, in the other test, 92° C. The results of four tests with juice diluted 1: 20 and containing two to four per cent gelatin are given in table 7. In three of these tests, the thermal death point was found to be 88° C., and in one test it was 87° C. for ten minutes. The thermal death points of tobacco-mosaic virus in solutions containing two to four per cent gelatin were identical with those in similar solutions that did not contain gelatin. It is concluded that the addition of this quantity of gelatin has no detectable influence upon the thermal death point.

THERMAL DEATH POINT: ONE-MINUTE EXPOSURE

In all the experiments reported above, a ten-minute interval was taken as a standard in determining the thermal death point. It is of interest to know the thermal death point of tobacco-mosaic virus for a one-minute

TABLE 5.—*Effect of exposure of tobacco-mosaic virus (diluted 1:20 with water and filtered through Berkefeld "N" filter candle) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
76	.....	13[a]	36	14	63	21
77	.....	20	27	36	83	28
78	41	21	11	10	83	21
79	20	12	5	19	56	14
80	15	5	11	5	36	9
81	14	9	14	8	45	11
82	7	9	4	7	27	7
83	2	4	3	6	15	4
84	0	0	0	1	1	0.25
85	0	0	0	0	0	0
86	0	0	0	0	0	0
87	0	0	0	0	0	0
Unheated controls	121	72	127	94		
		35	51	144	822	91
		58	120			

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

TABLE 6.—*Effect of exposure of tobacco-mosaic virus (in undiluted juice containing four per cent gelatin) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Total	Average
88	2246[a]	360	2606	1303
89	775	60	835	418
90	262	33	295	148
91	42	1	43	22
92	5	0	5	3
93	0	0	0	0
94	0	0	0	0
95	0	0	0	0
Unheated controls	2250	1619	3869	1935

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

exposure. In order to determine this, tests were made with undiluted juice of mosaic-diseased tobacco plants and with juice diluted 1:20 with water. Results of these tests are described below.

Undiluted juice. The results of four tests with undiluted juice are given in table 8. In two of these tests, the thermal death point for a one-

TABLE 7.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water and containing four per cent gelatin) to various temperatures for ten minutes*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
81	44[a]	132	179	19	374	94
82	21	260	210	40	531	133
83	26	390	124	19	559	140
84	22	52	88	24	186	47
85	7	141	25	8	181	45
86	4	26	11	4	45	11
87	1	9	0	2	12	3
88	0	0	0	0	0	0
89	0	0	0	0	0	0
90	0	0	0	0	0	0
91	0	0	0	0	0	0
Unheated controls	49	519	193	220	981	245

[a] Figures represent numbers of lesions produced upon 5 leaves of *Nicotiana glutinosa* by inoculation with virus sample indicated.

minute exposure was found to be 95° C.; in the other two tests, it was 96° C. Considerable inactivation of virus occurred at 92° C. in all four cases, and there was a gradual diminution in virus concentration with rise in temperature. The results indicate, however, that there is a sharper end point in the case of a one-minute exposure than in the case of a ten-minute exposure.

Juice diluted 1: 20 with water. Four tests were made in order to determine the effect of dilution on the thermal death point for a one-minute exposure. As in previous experiments, freshly extracted juice of mosaic-

TABLE 8.—*Effect of exposure of tobacco-mosaic virus to different temperatures for one minute*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
92	867[a]	3600	1280	2108	7855	1964
93	338	747	324	1870	3279	820
94	25	251	179	404	859	215
95	0	0	43	3	46	12
96	0	0	0	0	0	0
97	0	0	0	0	0	0
98	0	0	0	0	0	0
Unheated controls	7360	10330	11800	10880	40370	10093

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 9.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to various temperatures for one minute*

Temperature in degrees C.	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
87	1536[a]	648	408	479	3071	768
88	701	336	249	136	1422	356
89	528	71	128	128	855	214
90	208	93	92	11	404	101
91	16	5	6	1	28	7
92	0	0	0	0	0	0
93	.....	0	0	0	0	0
Unheated controls	8600	4400	3306	3516	19822	4956

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 10.—*Effect of exposure of tobacco-mosaic virus to a temperature of 90° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
20 min.	829[a]	7	179	1015	338
30 "	11	1	110	122	41
40 "	30	0	88	118	39
50 "	1	0	57	58	19
60 "	2	0	3	5	2
70 "	0	0	4	4	1
80 "	0	0	0	0	0
Unheated controls	9330	5688	10800	25818	8606

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 11.—*Effect of exposure of tobacco-mosaic virus to a temperature of 85° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
4 hrs.	416[a]	1500	243	2159	720
8 "	64	230	631	925	308
12 "	164	185	408	757	252
16 "	48	6	80	134	45
20 "	9	7	8	24	8
24 "	8	5	24	37	12
28 "	4	0	1	5	2
32 "	0	0	0	0	0
36 "	–	0	–	0	0
Unheated controls	6864	7822	5500	20186	6729

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 12.—*Effect of exposure of tobacco-mosaic virus to a temperature of 80° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
1 day	1112[a]	6688	1528	3088	3422	15838	3168
2 days	705	1048	1360	2747	5244	11104	2221
3 "	184	1545	805	2096	1728	6358	1272
4 "	133	444	312	680	860	2429	486
5 "	84	374	112	427	.....	997	249
6 "	18	280	35	640	193	1166	233
7 "	19	78	11	201	101	410	82
8 "	8	19	4	99	89	219	44
9 "	1	6	2	17	21	47	9
10 "	0	0	0	6	23	29	6
11 "	.....	.....	3	1	0	4	1
12 "	.....	.....	0	0	0	0	0
13 "	.....	.....	.....	0	0	0	0
14 "	.....	.....	.....	0	.....	0	0
15 "	.....	.....	.....	0	.....	0	0
Unheated controls	13300	11400	8000	10600	9152	52452	10490

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 13.—*Effect of exposure of tobacco-mosaic virus to a temperature of 75° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
10 days	1466[a]	1866	1600	4932	1644
15 "	263	192	33	488	163
20 "	21	19	20	60	20
25 "	17	11	7	35	12
30 "	21	8	5	34	11
35 "	3	6	0	9	3
40 "	0	0	0	0	0
40 "	0	0	0	0	0
Unheated controls	6044	3916	5964	15924	5308

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

diseased tobacco plants diluted 1:20 with water was used. Results of these four tests are presented in table 9. In all four tests, the thermal death point for a one-minute exposure was found to be 92° C. Partial inactivation occurred at 87° C., the lowest temperature tested.

THERMAL DEATH TIME FOR VARIOUS TEMPERATURES

Undiluted juice. The thermal death time for virus in freshly extracted juice of mosaic-diseased tobacco was determined for temperatures of 90°,

TABLE 14.—*Effect of exposure of tobacco-mosaic virus to a temperature of 68° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Total	Average
4 hrs.	11260	7377	.....	.....	.....	18637	9319
12 "	5250	3700	.....	.....	.....	8950	4475
24 "	7000	4600	.....	.....	.....	11600	5800
2 days	5000	3275	.....	.....	.....	8275	4138
3 "	5350	3303	.....	.....	.....	8653	4327
5 "	2369	811	.....	.....	.....	3180	1590
8 "	4450	1334	.....	.....	.....	5784	2892
10 "	2525	1045	.....	.....	.....	3570	1785
14 "	1635	655	.....	.....	.....	2290	1145
18 "	.....	.....	.....	.....	189	189	189
19 "	905	421	.....	.....	.....	1326	663
20 "	.....	.....	.....	.....	91	91	91
31 "	.....	.....	84	85	.....	169	85
33 "	.....	.....	185	.....	.....	185	185
37 "	.....	.....	.....	.....	12	12	12
39 "	.....	.....	52	.....	8	60	30
40 "	.....	.....	45	.....	.....	45	45
42 "	.....	.....	24	.....	.....	24	24
44 "	.....	.....	.....	.....	0	0	0
46 "	.....	.....	.....	.....	2	2	2
48 "	.....	.....	.....	5	.....	5	5
50 "	.....	.....	.....	5	0	5	3
52 "	.....	.....	0	2	1	3	1
53 "	5	0	.....	.....	.....	5	3
54 "	.....	.....	0	1	0	1	1
56 "	.....	.....	0	37	.....	37	19
58 "	.....	.....	0	4	.....	4	2
60 "	.....	.....	.....	26	.....	26	26
62 "	.....	.....	.....	9	.....	9	9
64 "	.....	.....	.....	5	.....	5	5
66 "	.....	.....	.....	4	.....	4	4
68 "	.....	.....	.....	11	1	12	6
70 "	0	0	.....	4	1	5	1
Unheated controls	17400	17800	.....	.....	.....	.....	.....
	9100	7400	5760	8280	13300	98440	10938
	10600	8800	.....	.....	.....	.....	.....

85°, 80°, 75°, and 68° C. In all cases the virus concentration remaining after heating was measured by inoculation of leaves of Early Golden Cluster beans. Results of tests at these temperatures are shown in tables 10 to 14. Table 10 shows the results of three tests with virus samples held at 90° C.; table 11, the results of three tests at 85° C.; table 12, the results of five tests at 80° C.; table 13, the results of three tests at 75° C.; and

TABLE 15.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 85° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
20 mins.	.....	3[a]	5	114	122	41
30 "	2	0	0	18	20	5
40 "	1	0	2	12	15	4
50 "	1	1	5	10	17	4
60 "	0	0	0	1	1	1
70 "	0	0	0	0	0	0
80 "	0	.....	0	0	0	0
Unheated controls	8600	4155	4225	3200	20180	5045

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 16.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 80° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
1 hr.	2014[a]	430	399	146	2989	747
2 hrs.	493	206	147	200	1046	261
3 "	132	171	291	141	735	184
4 "	76	58	107	45	286	72
5 "	91	93	194	11	389	97
6 "	6	12	220	9	247	62
7 "	5	94	123	2	224	56
8 "	1	12	48	1	62	16
9 "	0	7	45	0	52	13
10 "	5	9	9	0	23	6
11 "	0	0	2	4	6	2
12 "	0	0	1	0	1	0.25
13 "	0	0	0	0	0	0
14 "	0	0	0	0	0	0
15 "	0	1	0	0	1	0.25
16 "	0	0	0	0	0	0
Unheated controls	2014	4186	4784	5500	26990	3856
	4600	2122	3784			

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

table 14, the results of five tests at 68° C. It is concluded from these tests that tobacco-mosaic virus in undiluted juice is completely inactivated when held at 90° C. for 80 minutes, at 85° C. for 32 hours, at 80° C. for 12 days,

TABLE 17.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 75° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Total	Average
12 hrs.	990[a]	100	512	233	1835	459
24 "	355	4	184	40	583	146
36 "	4	0	20	1	25	6
48 "	0	3	68	0	71	18
60 "	0	0	2	0	2	1
72 "	0	0	0	0	0	0
84 "	.....	.....	0	0	0	0
Unheated controls	2448	2915	1228	1511	8102	2026

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

TABLE 18.—*Effect of exposure of tobacco-mosaic virus (diluted 1: 20 with water) to a temperature of 68° C.*

Time of exposure	Test No. 1	Test No. 2	Test No. 3	Total	Average
2 days	.....	906[a]	1070	1976	988
3 "	455	656	456	1567	522
4 "	235	354	576	1165	388
5 "	114	.....	.....	114	114
6 "	171	293	426	890	297
7 "	624	49	62	735	245
8 "	144	61	135	340	113
9 "	37	92	322	451	150
10 "	3	107	19	129	43
11 "	2	2	624	628	209
12 "	167	11	260	438	146
13 "	22	67	394	483	161
14 "	0	0	7	7	2
15 "	5	9	11	25	8
16 "	0	0	0	0	0
17 "	0	5	17	22	7
18 "	0	0	13	13	4
19 "	0	1	8	9	3
20 "	0	0	0	0	0
Unheated controls	3912	760	356	5028	1676

[a] Figures represent numbers of lesions produced upon 16 leaves of Early Golden Cluster beans by inoculation with virus sample indicated.

or at 75° C. for 40 days. It was not completely inactivated when held at a temperature of 68° C. for as long as 70 days.

Diluted juice. Tests were made to determine the thermal death time at various temperatures for virus in juice of mosaic-diseased tobacco diluted

1:20 with water. Tables 15 to 18 show the number of lesions produced on Early Golden Cluster beans by inoculation with virus heated to these temperatures. Table 15 shows results of four tests with diluted juice held at 85° C.; table 16, results of four tests at 80° C.; table 17, results of four tests at 75° C.; and table 18, results of three tests at 68° C. These results show that virus in freshly extracted juice, diluted 1:20 with water, is completely inactivated when heated to 85° C. for 70 minutes, to 80° C. for 13 hours, to 75° C. for 72 hours, or to 68° C. for 20 days.

CURVES SHOWING RATE OF INACTIVATION

Some of the data obtained in this investigation are plotted in the form of curves and presented in figures 1 and 2. Figure 1 shows the rate of inactivation of tobacco-mosaic virus in undiluted juice held at a temperature of 68° C. Data from which this curve was drawn were taken from table 14. The curve shows clearly the gradual diminution in virus concentration due to heating.

The curves in figure 2 were drawn with time plotted on a logarithmic scale and temperature on an arithmetic scale. They show the time-temperature relation for complete inactivation of virus. The points on the curves represent the times, shown in tables 1 to 3 and 8 to 18, for complete inactivation of virus at the temperatures indicated. With these curves, it is possible to estimate the thermal death time for any temperature between 68° C. and 96° C., or to calculate the thermal death temperature for any period between one minute and 40 days. Although the curve for inactivation of virus in undiluted juice and that for virus in juice diluted 1:20 are similar in shape, they are closer together at the higher temperatures tested than at the lower. This appears to show that dilution alters the thermal death time more at low temperatures than at high temperatures.

DISCUSSION

The results obtained in this investigation are in fairly close agreement with those of other workers. The seemingly higher thermal death point obtained by the writer and also by Johnson and Grant (8) may be due to the use of the rubbing method of inoculation, which allows detection of minute quantities of virus. It has been shown that the thermal death point is influenced by concentration of virus and by concentration of solids in the dispersing medium. It may be that such factors would be operative in the case of virus samples obtained from host plants grown under different environmental conditions. Differences between results observed by various investigators, as well as differences between certain samples of virus used by the writer, may possibly be due to differences in plants from

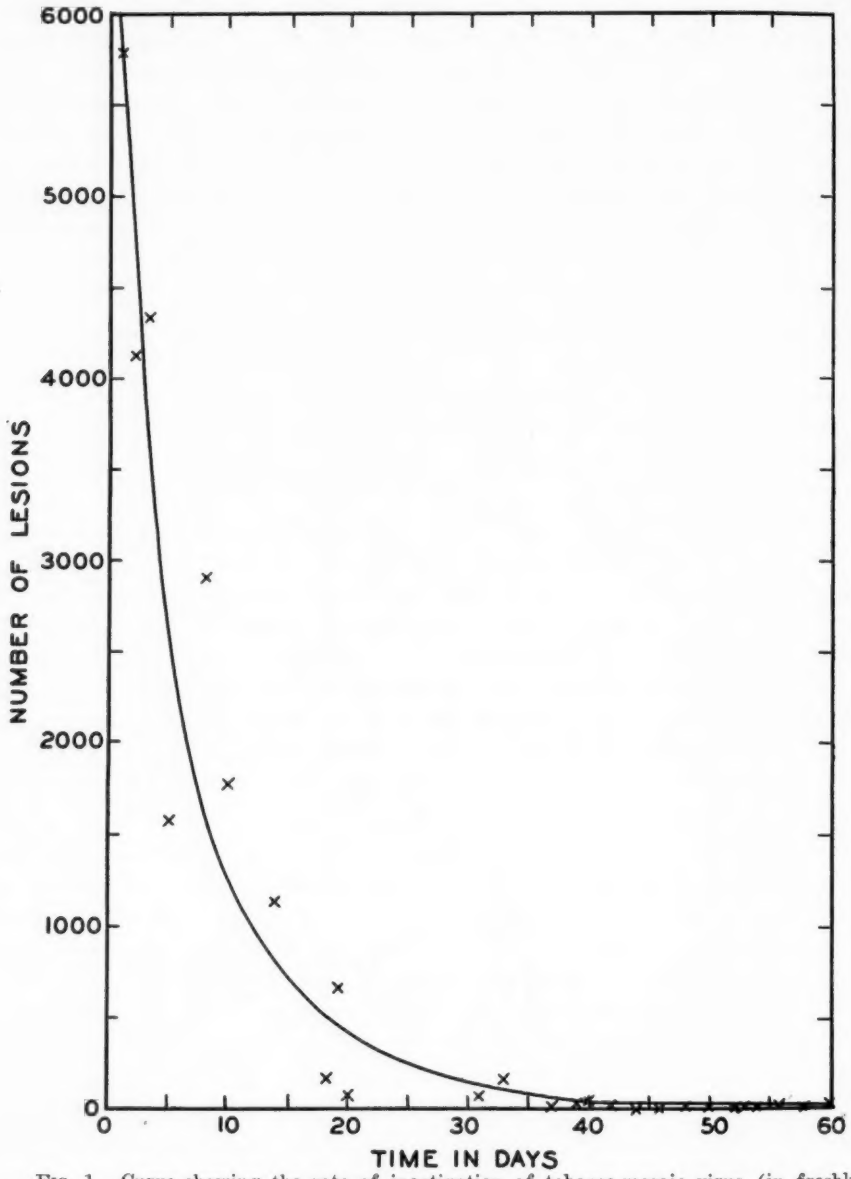

FIG. 1. Curve showing the rate of inactivation of tobacco-mosaic virus (in freshly expressed juice of diseased tobacco plants) when held at a temperature of 68° C. The points on the curve show the average number of lesions produced on leaves of Early Golden Cluster beans by inoculation with virus samples heated for the length of time indicated.

which the virus samples were obtained. It is of importance that such factors as concentration of solids and nature of the dispersing medium be taken into consideration when comparing the thermal death points of different viruses or even of the same virus from different sources.

Another factor that may account for some of the variation obtained in different tests with tobacco-mosaic virus is the difficulty of maintaining a uniform temperature over a considerable period of time. Examination of the curves presented in figure 2 shows that a slight change in temperature causes a significantly large change in thermal death time of the virus. Variation in temperature of as much as one degree C. might result in a considerably shorter or considerably longer thermal death time.

Thermal death-time curves for several thermophilic organisms were published by Bigelow (3). These curves, on semilogarithmic paper, are straight lines except at temperatures below 105° C., where they tend to turn downward. It is of interest that the thermal death-time curves of tobacco-mosaic virus (Fig. 2) resemble those shown by Bigelow for thermo-

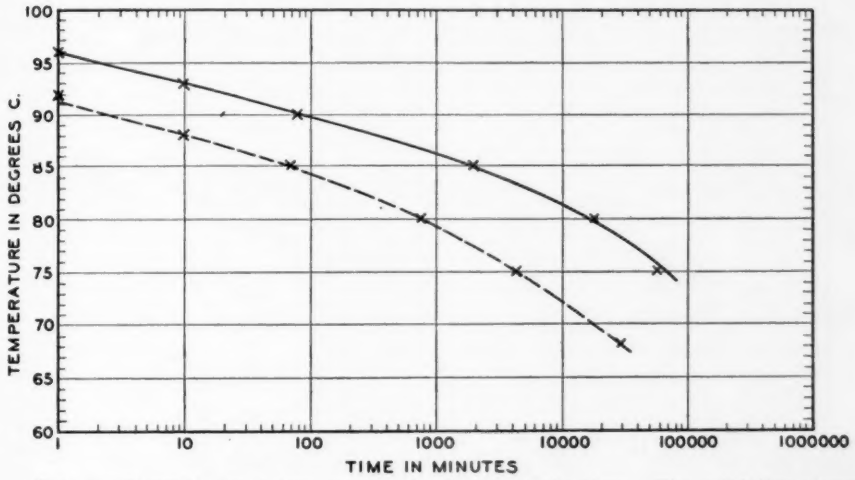

FIG. 2. Thermal death-time curves of tobacco-mosaic virus. The solid line shows the time required for complete inactivation of virus in freshly expressed juice of mosaic-diseased plants at the temperatures indicated; the broken line, the time required for inactivation of virus in juice diluted 1: 20 with water.

philic organisms. These curves for tobacco-mosaic virus are almost straight lines at the high temperatures but bend downward sharply at temperatures below 80° and 85° C.

The virus of tobacco mosaic is more resistant to heat than most of the other plant viruses that have been studied up to the present time. No other plant virus is known to have a higher thermal death point. That it

is far more resistant than are most vegetative bacteria is shown by its ability to withstand the temperature of pasteurization for many days.

SUMMARY

The local-lesion method for measuring virus concentration was used in a quantitative study of the rate of inactivation of tobacco-mosaic virus by heat. It was found that virus in undiluted juice of mosaic-diseased tobacco plants was inactivated in one minute at 96° C., in ten minutes at 93° C., in 80 minutes at 90° C., in 32 hours at 85° C., in 12 days at 80° C., and in 40 days at 75° C. It was not completely inactivated when held for 70 days at 68° C. Virus in juice of mosaic-diseased tobacco plants diluted 1:20 with water was completely inactivated in one minute at 92° C., in ten minutes at 88° C., in 70 minutes at 85° C., in 13 hours at 80° C., in 72 hours at 75° C., and in 20 days at 68° C.

Tables giving measurements of virus concentration after heating for different periods of time at the same temperature and for different temperatures for the same length of time are presented in the text. These measurements indicate that inactivation of tobacco-mosaic virus by heat is a gradual process that goes on more rapidly at first than after partial inactivation has occurred.

Time-temperature curves for inactivation of mosaic virus in undiluted juice and in juice diluted 1:20 with water were drawn on semilogarithmic paper and are presented in the text. They show the time required for complete inactivation of virus at temperatures between 68° and 96° C.

Thermal death-point determinations made with virus in juice diluted with water and in juice diluted with extract of healthy tobacco indicate that inactivation of virus is influenced by concentration of virus and concentration of solids in the dispersing medium.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, PRINCETON, NEW JERSEY.

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LEAF SCORCH OF NARCISSUS[1]

DON B. CREAGER

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A disease that causes premature death of narcissus leaves was brought to the attention of the writer in the summer of 1929 (Fig. 1). In general,

FIG. 1. Narcissus leaves (var. Sir Watkin) destroyed by leaf scorch; natural inoculation.

it appears that the *Leedsii* and *Polyanthus* types of narcissus are most severely affected, but the disease is known to occur also on nearly all of the other types. Among the commercial varieties commonly infected are, Sir Watkin, King Alfred, White Lady, Queen of the North, Laurens Koster, Orange Cup, Early Perfection, and Victoria. The disease occurs also on

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the leaves and scapes of amaryllis (hybrids of *Hippeastrum vittatum* Herb.).

Fields or portions of fields of narcissus in which the foliage is severely affected have a burned or scorched aspect, which has given rise to the name "leaf-scorch," as proposed by British phytopathologists, and adopted here.

Leaf scorch was first reported by Aderhold (1) in 1900 on narcissus in Germany, while, apparently, the disease on amaryllis was first noted by Dougherty (3) in 1916. For over a decade the diseases on these two susceptes were not thought to be related, but in 1929, Smith (11) described observations and experiments which indicated that the disease on narcissus was the same as that on amaryllis. Recent experiments by Feekes (4) gave similar indications.

Leaf scorch has been reported from many places in western Europe, and from the United States and Canada; its distribution is probably coextensive with that of narcissus and amaryllis culture.

The disease is responsible for considerable damage in large fields of narcissus grown for the flower and bulb market, since the leaves are killed 4 to 8 weeks before they normally die. The actual loss resultant from premature death of the leaves has not been determined experimentally, but growers estimate that the loss in weight of bulbs may be as much as 30 per cent or more. Since all food normally stored in the bulb is elaborated in the green leaves, this reduction is attributable to partial starvation caused by the premature death of the foliage. Ordinarily the disease on narcissus does not assume epiphytotic proportions until after the flowering period, so that the flowers usually escape infection. In southern regions, however, the buds and flowers also may be damaged.

It seems that no comprehensive study of leaf scorch has ever been made. Reports of preliminary studies have been published, but even in these the general characteristics of the disease and its causal organism are inadequately described. It is the purpose of this paper to present results of observations and experiments as a contribution to a more complete understanding of the disease and the pathogen as it occurs on narcissus in the eastern United States.

SYMPTOMS

The initial symptoms of narcissus leaf scorch appear very early in the season as yellowish, reddish, or brownish lesions at the tips of the first few leaves (Fig. 2, A). These lesions may be evident as soon as the tips have reached the surface of the soil, or they may not appear for some time thereafter. Typically the lesion is confined to the upper 2 or 3 inches of the leaf (Fig. 2, B). The older parts of the lesion soon become necrotic and often zonate in appearance. The transitional area between healthy and

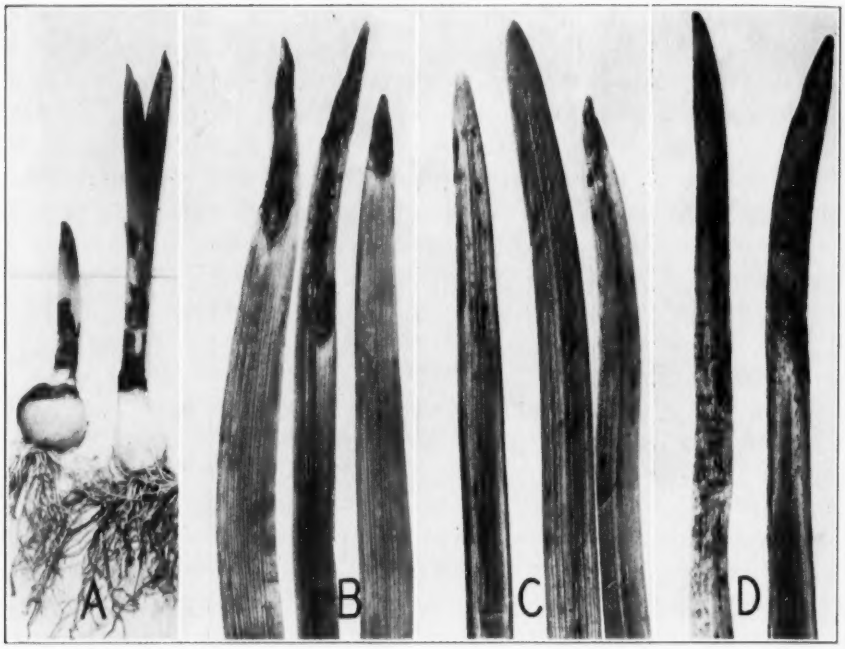

FIG. 2. Symptoms of narcissus leaf scorch. A. Primary lesions at the tips of very young leaves and sterile sheaths (horizontal line represents soil surface). B. Primary lesions at tips of older foliage leaves. C. Secondary lesions initiated by inoculum from primary lesions, leaf to the right bearing primary lesion at tip and secondary lesions below. D. Appearance of secondary lesions after leaves have been killed by the disease, showing the dark reddish brown scab-like spots as they appear against the lighter grayish brown tissue surrounding them.

diseased tissue is typically yellow. Since these lesions at the tips of the leaves are the first to appear, they may be called primary lesions, while those brought about by inoculum from primary lesions may be called secondary lesions.

Secondary lesions first appear as minute water-soaked spots characterized by yellow centers, as they age. Soon the centers become reddish brown and necrotic as the area of the lesions expands (Fig. 2, C). Secondary lesions rarely measure over $\frac{1}{8}$ in. $\times \frac{1}{2}$ in. and usually remain much smaller. The spots may be so numerous and close together as to coalesce. Eventually the tissue surrounding these spots dies and becomes light grayish brown. Distributed in this dead tissue, the spots appear as somewhat raised, scabby, dark reddish brown areas (Fig. 2, D). These secondary lesions cause the greater part of the damage because of their frequency and common occurrence on the lower portions of the leaves. The leaves die very quickly after the appearance of secondary lesions.

Numerous dark pycnidia, which serve as signs of the disease, project through the surface of the necrotic tissue. They are abundantly produced, usually in both primary and secondary lesions proper, as well as in the light grayish brown, dead tissue surrounding the lesions.

Since the symptoms of leaf scorch and those of another common disease known as "fire," caused by *Botrytis* spp., are somewhat similar in that both cause premature death of leaves, they may be confused. Moreover, both may occur in the same planting and even on the same leaf. It is believed, however, that they may be distinguished with some degree of accuracy by the presence of the reddish brown, scab-like spots in the dead portions of infected leaves in the case of leaf scorch, and by the absence of the scab-like spots in the case of fire. The presence of pycnidia in the necrotic areas in leaf scorch and the fuzzy grayish masses of conidiophores in Botrytis fire are also of diagnostic value.

THE PATHOGEN

Name and Classification. Leaf scorch is caused by the fungus *Stagonospora curtisii* (Berk.) Sacc. It appears that the fungus was first collected by Curtis, at Society Hill, South Carolina. It was named by Berkeley and, in 1878, described by Cooke (2). Berkeley gave it the name of *Hendersonia curtisii.* The description given by Cooke is as follows:

Hendersonia curtisii Berk. in herb. Curt. Sporis elongato-ellipsoides demum biseptatis, 0.017–0.021 by 0.0075 mm. Ad narcissum.

Berkeley, also, established the genus Hendersonia, and in the same paper Cooke gives the following description:

Hendersonia Berk. Ann. Nat. Hist. Perithecia carbonacea, sporis elongatis, bi vel multiseptatis, hyalinis vel coloratis, primo pedicellatis demum liberis (Sporocladus corda proparte).

In 1887, Saccardo (9) separated Berkeley's genus Hendersonia into two groups of generic rank on the basis of spore color. The species with hyaline spores constituted the new genus Stagonospora, while those with colored spores were retained in Hendersonia. Saccardo, therefore, lists the leaf-scorch fungus as *Stagonospora curtisii* (Berk.) Sacc.

A very scant specimen collected at Society Hill, South Carolina, by Curtis is in the Curtis herbarium at Harvard University. The collection is so meager that proper study and comparisons could not be made. Sketches of spores on the specimen packet indicate that they consist of 1 to 3 cells and resemble the spores isolated from narcissus leaves affected with scorch. Since no note accompanied these sketches, it could not be determined by whom they were made. These sketches and the brief description by Cooke indicate that *Hendersonia curtisii* Berk. is the same organism that has proved to be the cause of the leaf-scorch disease.

In 1900 Aderhold (1) isolated a fungus from narcissus leaves in Germany, which he named *Phyllosticta narcissi,* n. sp. Upon the same leaves he also found spores of a fungus resembling those of *Stagonospora curtisii.* Since it is now known that the spores of the leaf-scorch fungus are so variable, indications are that Aderhold did not have 2 distinct fungi, but one and the same organism. The description of the diseased leaves from which he isolated *P. narcissi* also indicates that he was dealing with leaf scorch.

In recent studies Smith (11) and Feekes (4) recognized the organism as *Phyllosticta narcissi* Aderh. but suggested the possibility of its being the same as *Stagonospora curtisii.*

A comparative study of the descriptions of *Phyllosticta oudemansii* by Saccardo (10) and *Stagonospora narcissi* by Hollos (5) occurring on narcissus, and *Phyllosticta* sp. by Dougherty (3), *P. gemmipara* by Zondag (12), *Phoma amaryllidis* by Kotthoff and Friedrichs (7), and *Stagonospora* sp. by Petrak (8), occurring on amaryllis, has led the writer to believe all are identical and are like the narcissus leaf-scorch fungus.

These differences in opinion concerning the identity of the pathogen very probably have been due to its occurrence on two different hosts and the variability in size and spore septation. Because of priority the proper name of the organism is, therefore, *Stagonospora curtisii* (Berk.) Sacc.

Pathogenicity. The pathogen was isolated from leaves, buds, flowers, and bulb scales. Isolations were made from the small reddish brown spots in green leaves, as well as from dead leaves bearing pycnidia. The organism was isolated also from the leaf sheaths near the soil surface, as well as from the outer scales of bulbs.

The pathogenicity of *Stagonospora curtisii* has been established by inoculating narcissus leaves with mycelium and spores. A number of freshly wounded and sound leaves of the variety Sir Watkin, growing in the greenhouse, were inoculated with mycelium from cultures on potato-dextrose agar. In several cases a piece of agar containing the mycelium was placed near the bases of the older leaves so that such young leaves as emerged subsequently might come in contact with the fungus. Leaves of the check plants were wounded or were left unwounded, and sterilized agar was placed in the wounds and on the uninjured parts. Bell jars were placed over all of the plants to maintain a high humidity.

Within 24 hours the first symptoms of disease became evident as water-soaked areas around the wounds. In 48 hours the centers of the lesions had become necrotic and brown and were surrounded by a yellowish area almost an inch in length. At the same time yellow areas appeared on the side opposite the inoculum on the sound leaves. In 3 days numerous pycnidia appeared in the dead areas surrounding the wounds and the

lesions extended much farther. The lesions of the sound inoculated leaves had also enlarged and were necrotic in the centers. After a week the bases of the older leaves and the tips of younger leaves that came in contact with the blocks of agar containing the organism exhibited typical leaf-scorch lesions. Twenty days after inoculation the leaf tips on the inoculated plants were dead and dry, while those of the check plants were perfectly healthy (Fig. 3, A). A repetition of these experiments in the greenhouse and in the field gave similar results.

A suspension of spores in distilled water was atomized on leaves of a variety of *Narcissus poeticus* growing in the field. A large moist chamber was placed over the inoculated plants and another was placed over plants near-by to serve as a check. The chambers were removed after 48 hours. In 4 days numerous reddish brown spots were faintly apparent on the leaves sprayed with spores, while the check plants were entirely free from spots. After 10 days the inoculated leaves exhibited the typical spots resembling the secondary lesions that occur naturally in the field and greenhouse. Four weeks after inoculation nearly every leaf in the spore-sprayed plot was dead, shriveled, and dry, while the leaves in the check plot were free from infection (Fig. 3, B and C). This experiment, when duplicated, yielded similar results.

A comparative study of the pathogenicity of the 2 organisms isolated from amaryllis and narcissus, respectively, has been made. The fungus from both sources produced the typical red spots on the leaves of amaryllis and the typical leaf-scorch symptoms on narcissus. These results and observations verify the conclusions of Smith (11) and Feekes (4), *i.e.*, that one and the same organism is responsible for the disease on the 2 suscepts.

Life history. All evidence available suggests that the fungus overwinters in or on the bulbs, and that this is the principal source of inoculum for the primary cycle. The organism has not been isolated from bulbs a sufficient number of times to establish this point beyond a doubt, but the following observations and results of experiments tend to support this probability.

Ninety narcissus bulbs of the variety Queen of the North, whose leaves were destroyed prematurely by the leaf-scorch pathogen during the previous season, were taken from Long Island, New York, to Durham, North Carolina. They were planted in soil in which narcissus had never been grown. The next season over 100 leaves bore primary lesions. This result confirms observations of experienced growers and indicates that the pathogen is carried on the bulbs. In another experiment, 90 bulbs of the variety Laurens Koster were used, the leaves of which had also been destroyed prematurely by the leaf-scorch pathogen during the previous season. One-

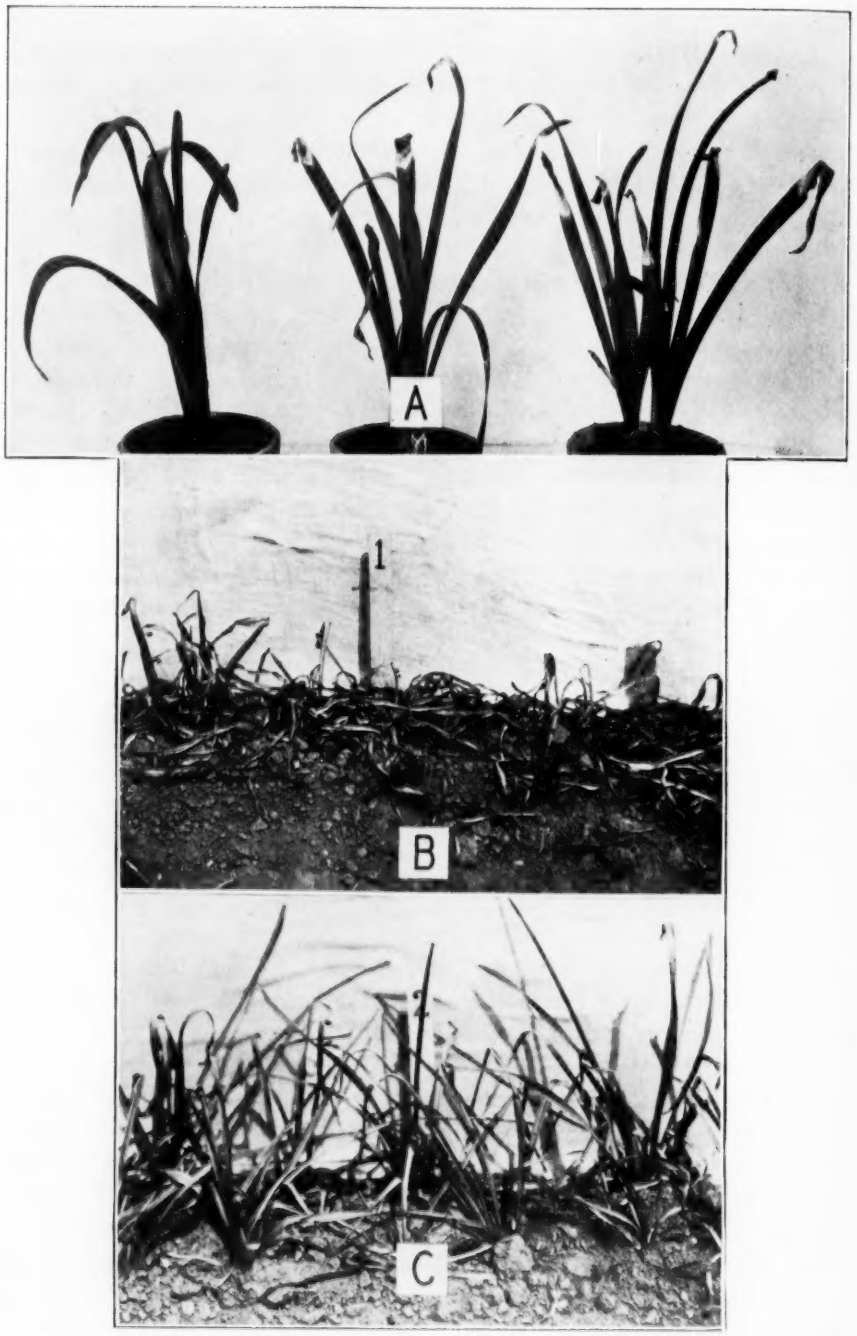

FIG. 3. Results of inoculation experiments. A. Narcissus leaves killed at the tips as a result of inoculation with mycelium. Check plant at the left. B. Result of inoculation with spore suspension, photographed four weeks after inoculation. C. Plants used as a check against those inoculated in B, leaves beginning to die naturally because of old age but entirely free from leaf scorch.

half of this number were treated for 2 hours in one-half per cent Formalin solution immediately before planting, while the remaining 45 bulbs were not treated. Each lot was planted in a row about 2 feet from each other in soil in which narcissus had never been grown. The following season 18 leaves of the nontreated lot exhibited typical primary lesions and by the end of May 62 leaves were infected, while not a single primary lesion appeared in the lot treated with Formalin. These results indicate that the fungus is on or in the tissue of the bulb scales, since infection occurred in leaves of the nontreated lot and not in those treated with Formalin.

It has not, however, been definitely proved that the fungus overwinters on the bulbs nor are the form and exact location of the fungus during the dormant period definitely known. This is especially true of bulbs grown in accordance with present commercial practices, but, under rather unusual conditions, pycnidia and mycelium of the pathogen were found growing in the outer scales in the neck region of 2 bulbs. These bulbs, with necks and upper halves exposed, were growing in an old naturalized planting in an orchard near Norfolk, Virginia. In another instance the dead scales in the neck region of a bulb were broken off and had been carried upward in the form of a ring about the growing leaves. At the point of contact with the ring of dead tissue a primary lesion appeared on

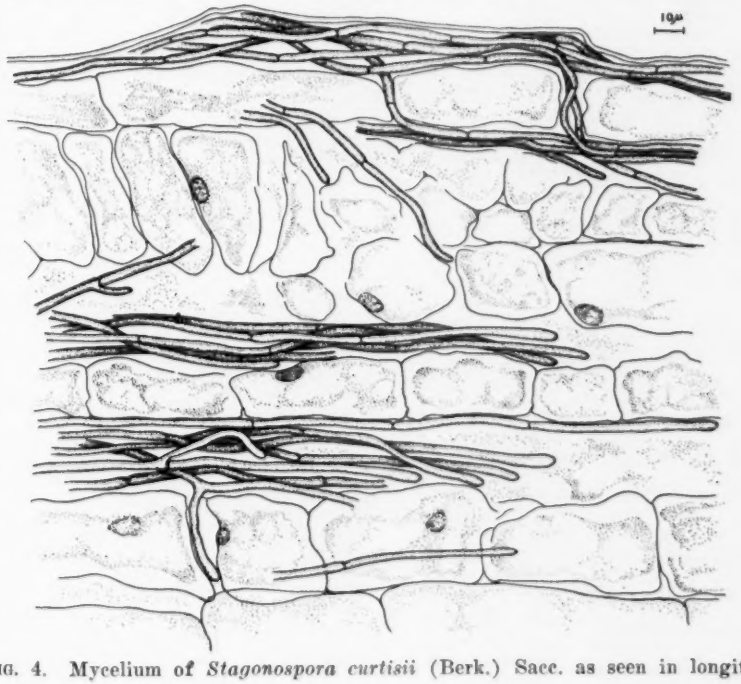

FIG. 4. Mycelium of *Stagonospora curtisii* (Berk.) Sacc. as seen in longitudinal section of narcissus leaf.

each leaf. Upon examining the dead scales, numerous pycnidia and mycelium of the leaf-scorch fungus were found.

According to Feekes (4), the fungus has been found as a loose, grayish, mycelial web over the outer surface and between the scales of dormant bulbs. He suggests that the fungus may survive the winter in this form. Thus far, the writer has never observed the mycelial web on any bulbs he has examined.

After the dormant period, the new leaves grow out of the bulb and come in contact with the dead basal portions of the previous season's foliage leaves in the neck region. It is believed that the tips of the new leaves are inoculated with the pathogen as they pass this region, since lesions are usually well advanced when the tips come through the surface of the soil, and only a few of the outer leaves bear primary lesions.

The primary lesions contain numerous intercellular hyphae of the pathogen, which not only grow between the cells of the mesophyll, but even penetrate between the epidermis and cuticle (Fig. 4). Commonly, a rather

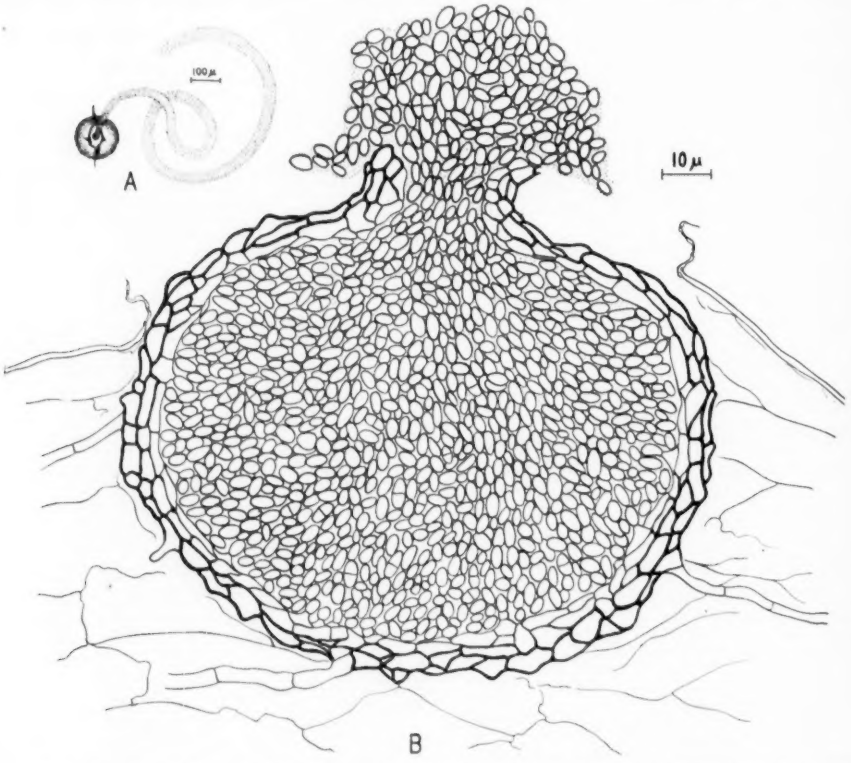

FIG. 5. Pycnidia of *Stagonospora curtisii* (Berk.) Sacc. A. Pycnidium as seen from above, showing the gelatinous matrix and spores oozing out in a cirrus. B. Median vertical section through a mature pycnidium produced in a living leaf in the greenhouse.

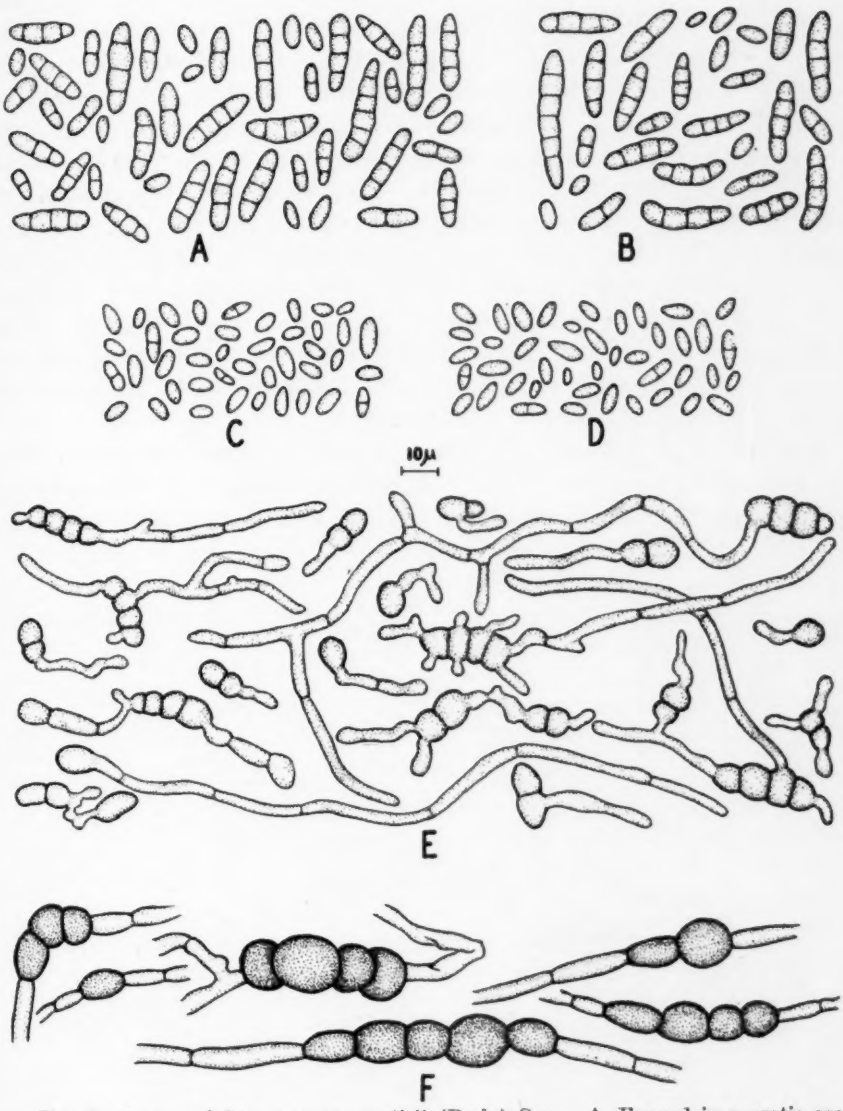

FIG. 6. Spores of *Stagonospora curtisii* (Berk.) Sacc. A. Formed in necrotic areas of leaves in the field following natural inoculation. B. Produced by an isolation from narcissus, in living leaves of narcissus in the field as a result of artificial inoculation. C. Produced by an isolation from narcissus in autoclaved narcissus leaves. D. Produced by an isolation from amaryllis in autoclaved narcissus leaves. E. Germinating spores. F. Thick-wall resting cells or chlamydospores.

distinct hyphal mat develops between the thick cuticle and the outer cell walls of the epidermis. In living tissue the progress of mycelial growth is relatively slow, but after the tissue dies growth is very rapid.

Pycnidia soon develop subepidermally in the dead tissue. They appear as minute brown or black, papillate, globose bodies. Each pycnidium opens by an ostiole at the leaf's surface through which multitudes of spores ooze out in cirrus formation. The spores are embedded in a gelatinous matrix and when the pycnidium becomes wet, water is imbibed by the gelatinous substance, causing it to swell greatly. As a result the matrix oozes out through the ostiole carrying with it all of the spores (Fig. 5). The principal agent of spore dissemination is water during rains. Splashing rain carries spores from the infected areas to healthy areas of the same leaf and to other leaves of the same and near-by plants. Wind may aid in disseminating spores during rains by carrying spore-laden droplets. Spores produced in the primary lesions constitute the inoculum that initiates the principal secondary cycles of infection.

The spores germinate in water within several hours (Fig. 6, E). The first obvious change is a marked swelling of each cell and, at the same time, the walls may become slightly olive brown. One to several septate germ

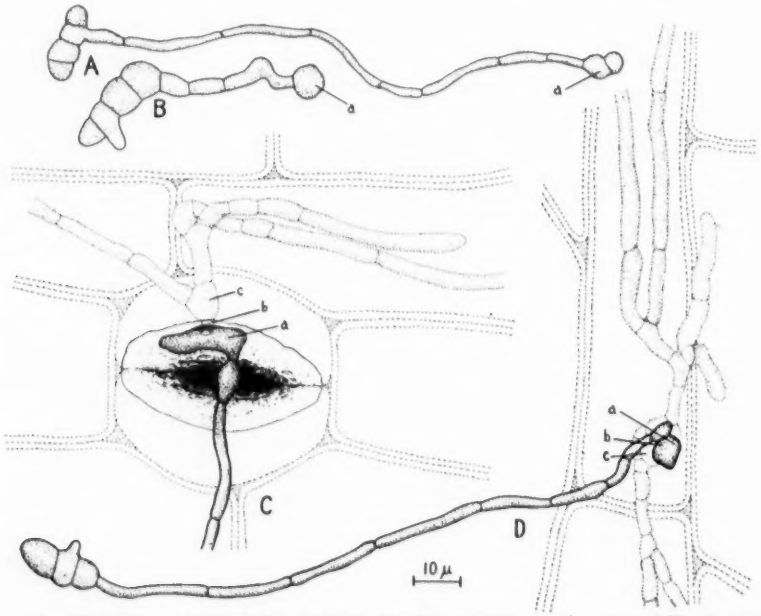

FIG. 7. Entrance of the pathogen into the tissue of the leaf. A and B. Appressoria (a) formed at the tips of germ tubes. C. Young mycelium invading the subcuticular region of the leaf, showing the appressorium (a) which formed against one of the guard cells of a stoma, the small invasion-hypha (b), which grew from the underside of the appressorium and penetrated the thick cuticle. D. Mycelium developing between cuticle and epidermis of a leaf, showing the appressorium (a) at the tip of the germ tube, the small invasion hypha (b), seen in vertical section, and the typical vesicle (c) from which the intercellular hyphae originated.

tubes develop from one cell, or from all cells of a spore. The germ tubes grow over the surface of the leaf and develop appressoria at their tips (Fig. 7, A and B). Soon a small invasion hypha develops from the underside of the flattened appressorium and penetrates the heavy cuticle. After this hypha grows through the cuticle and reaches the outer wall of the epidermal cells a more or less enlarged vesicle forms, which is sometimes composed of several cells. One to several hyphae grow from the vesicle and rapidly develop into an intercellular mycelium (Fig. 7, C and D). The present of the mycelium causes infection and a consequent secondary lesion.

Secondary cycles of infection also may be initiated by actual contact of diseased leaves and flower parts that bear primary lesions with healthy parts during damp and rainy weather.

Secondary lesions are formed abundantly over all parts of the leaves and are especially numerous near the base. The mycelium in the leaves grows downward toward the neck region of the bulb, as the leaves gradually die, and very probably remains alive in the remnants of dead leaves during the dormant period of the bulb. It has been demonstrated that a desiccated culture of the fungus can remain alive more than a year and that transfers will grow vigorously on fresh bulb agar.

Morphology. The intercellular mycelium in the succept tissue is composed of septate hyphae the branches of which usually originate immediately below the septa (Fig. 4). The hyphae in the margins of the lesions may be composed of relatively long cells with no apparent constrictions at the septa. In the older portions, however, they may be composed of relatively short and rounded cells with very prominent septal constrictions. The young hyphae are colorless and the older portions gradually become dark brown. The older hyphae, in moist tissue, may remain colorless or faintly colored for a relatively long time, but finally become darker.

The mycelium grows well on a number of agar media including potato dextrose, and extracts of bulbs of narcissus, tulip, iris, and onion, and of narcissus leaves. It has also been grown on autoclaved leaves of narcissus and amaryllis. The variations of mycelial growth on the different media are not marked. Since a very luxuriant growth developed on narcissus bulb agar, resembling that on autoclaved narcissus leaves, the characteristics as exhibited on that medium, only, will be described. When very small pieces of the mycelium were planted in Petri dishes maintained at room temperature (20 to 24° C.), mats of mycelium 3 to 5 cm. in diameter developed in 4 days. The mycelial mat was composed of both aerial and submerged hyphae. Aerial hyphae in mass are always some shade of gray, with usually an olive-green or olive-brown tint, and, with age, become dark brownish gray. The submerged hyphae in mass are dark olive brown

to black. The medium also becomes brownish to black, but is not so deeply colored as the submerged hyphae.

In autoclaved leaves, potato-dextrose agar, and narcissus-bulb-extract agar, thick-wall, dark, chlamydospore-like cells form abundantly in the older cultures. One to several adjacent cells, either terminal or intercalary, become enlarged, thick-wall and darker brown (Fig. 6, F). They serve as resting cells, as evidenced by the fact that under favorable conditions they give rise to new hyphae. Sometimes, by repeated division of these cells, sclerotium-like bodies are developed. It is believed that in some cases these same bodies function in pycnidial formation.

Pycnidia are globose to appressed-globose and ostiolate (Figs. 5, B, and 8, L). When crowded, or when for some other reason unequal pressure is exerted on their sides, they may be somewhat irregular. The mature bodies are 110 to 275 microns in diameter, the average being approximately 175 microns. The wall is composed of irregular, compressed, brownish cells, which form a layer 2 to 4 cells thick. The inner layer of cells is colorless, or nearly so, and the cell walls are much thinner than those toward the outside. The thin-wall cells that line the bottom and sides of the pycnidium project into the cavity and constitute the sporophores (Fig. 5, B).

The origin and development of pycnidia have been studied from growth on narcissus-bulb-extract agar and autoclaved leaves in Petri dishes. The best procedure for tracing pycnidium formation consisted in utilizing strips of epidermis attached to slides by means of the natural mucilage in the leaf tissue. These slides were placed in Petri dishes lined with several layers of water-soaked filter paper, which were then sterilized in the autoclave. A bit of mycelium was placed, aseptically, at one end of the epidermal strip. Within three days all stages of development from first indications of primordia to mature sporulating pycnidia could be observed in these epidermal strips. Cotton Blue in phenol and glycerine solution, when used as an *in toto* stain, was helpful in studying the early stages of pycnidial formation.

The origin and early development of pycnidia are rather variable (Fig. 8). Most commonly the primordium arises from a few adjacent cells of a main hypha. Several adjacent cells enlarge and repeatedly divide in all planes (Fig. 8, A–C). In the early stages they may appear identical with the chlamydospore-like cells previously described. Commonly, hyphal branches grow from the outer cells of the mass (Fig. 8, B–D). Some of these hyphae seem to take part in the development of the fruit-body, since they may branch and anastomose with the central mass of the primordium. Other branches, originating from cells of the main hypha some distance away from the developing pycnidium, may also anastomose with it (Fig.

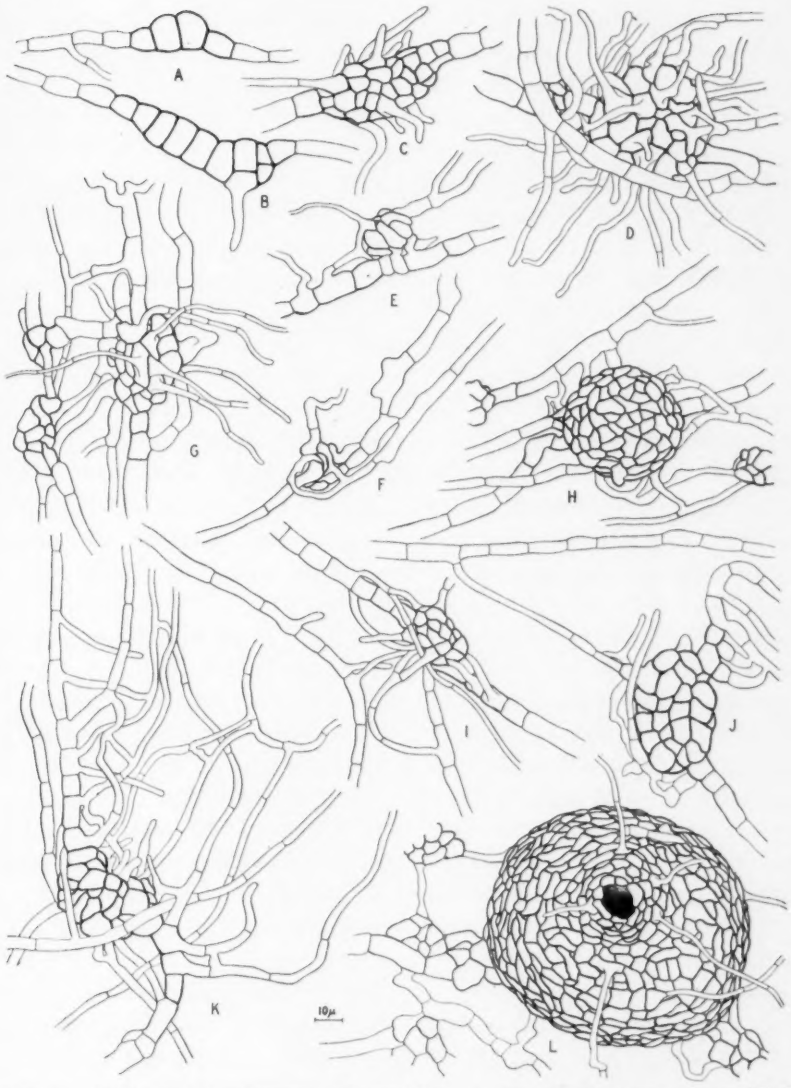

FIG. 8. Origin and development of pycnidia. A. Very early stage of pycnidial development. B. Slightly later stage. C. Multicellular pseudoparenchymatous stage with branches protruding from the mass. D. Later stage with branches protruding, some of which are rebranching, turning back and anastomosing with the primordium. E. Primordium formed by entwining and anastomosing of short branches at the side of a main hypha. F. Several branches entwining and anastomosing to form a primordium. G. Primordium in which several hyphae are involved. H. Similar condition in which branches from another main hypha have anastomosed with the primordium. I. Branches from the original hypha and from another main hypha near-by, anastomosing with the primordium. J. Similar condition, showing curious anastomoses of hyphal branches with the primordium, with one another, and with another hypha near-by. K. Hyphal branches from the primordium and cells of the original hypha near-by that have rebranched and anastomosed with the primordium and with one another to form an intricate network. L. Nearly mature pycnidium.

8, I). Hyphal branches from the primordium frequently anastomose with one another and other hyphae near-by (Fig. 8, G–K). The significance of anastomoses of these branches with one another and with other hyphae some distance away from the primordium proper can not be definitely explained, but they may perhaps aid in transportation of food from other hyphae to the developing pycnidium. Hyphal branches from main hyphae near-by have also been observed to branch and anastomose with cells of the primordium (Fig. 8, G–I). It is difficult to determine with certainty whether branches from other hyphae have anastomosed with the primordium, or whether they have grown from the primordium and fused with the near-by hyphae. There is some evidence that both conditions occur.

Another type of origin different from that described above has been observed in which short branches from the main hypha entwine and anastomose with one another, the primordium being formed at the side of the main hypha (Fig. 8, E). This method of origin is infrequent.

There is some indication that still another type exists in which several main hyphae branch, entwine, and anastomose with one another and gradually develop into the primordium by growth and multiplication of cells (Fig. 8, F).

According to Kempton (6), pycnidia originate and develop by 2 main methods, meristogenous and symphogenous. The meristogenous method resolves itself into 2 modes, simple and compound. In the simple mode the pycnidium develops from a single cell or a few adjacent cells of a single hypha; in the compound mode, adjacent cells of two or more continuous hyphae divide, swell, and sometimes branch, all of which then anastomose freely to form a pseudoparenchymatous mass. The type of origin of pycnidia of the narcissus leaf-scorch fungus is evidently a modification of the simple and compound meristogenous method, varying from one extreme to the other. This variable characteristic in pycnidial development is not uncommon among the members of the Sphaeropsidales.

Numerous spores are formed on the short sporophores and soon completely fill the cavity of the mature pycnidium (Fig. 5). They are exceedingly variable in size and septation, whether taken from an individual pycnidium or from pycnidia that have grown under different conditions (Fig. 6, A–C). Successive portions of mycelium from a culture were planted on narcissus-bulb agar, another on autoclaved narcissus leaves, another on living leaves of plants in the greenhouse under warm humid condition, and another on leaves in the field under normal conditions. Spores produced on bulb agar, autoclaved leaves, and in living leaves in the greenhouse were very abundant, but all were very small, and nearly all were unicellular, with bicellular ones occurring rarely (Fig. 6, C). The

spores taken from leaves in the field were much larger and the majority were quadricellular (Fig. 6, B). Bicellular spores also were present, while unicellular ones were relatively rare. The spores produced by artificial inoculations in the field were similar to those produced in lesions formed as a result of natural infections (Fig. 6, A and B). Examination of various collections from the field shows that all these variations of spores may occur. Smith (11) also has recognized the extreme variability of the spores of this fungus.

The spores are hyaline, elliptical to fusiform to nearly cylindrical, bluntly rounded at both ends, consisting of 1 to 6 cells, usually slightly constricted at the septa; spores of 1, 2, and 4 cells are common; those with 3, 5, and 6 cells, rare; size variable; unicellular spores, 4.5 to 10.2 by 3.2 to 4.8 microns, average 7.5 by 3.8 microns; bicellular spores 8.0 to 16.0 by 3.2 to 6.4 microns, average 11.5 by 4.5 microns; quadricellular spores 13.4 to 28.0 by 4.8 to 8.0 microns, average 21.0 by 6.5 microns.

SUMMARY

This investigation deals with leaf scorch of narcissus, a disease that kills part or all of the leaves of susceptible varieties 4 to 8 weeks before they should normally die. The disease also occurs on amaryllis.

The initial symptoms of the disease in narcissus appear as yellowish, reddish, or brown lesions that soon develop necrotic areas at the tips of the first leaves. Later in the season numerous secondary lesions appear as reddish brown spots on leaves near the primary lesions. It is the secondary lesions that effect the greater damage.

The disease is caused by the fungus *Stagonospora curtissii* (Berk.) Sacc.

Pathogenicity has been proved by inoculation with mycelium and with spores.

Experimental results and observations indicate that the fungus overwinters in or on the bulbs and that this is the principal source of inoculum for the primary cycle of infection.

Numerous pycnidia are produced in necrotic areas of the lesions. Their origin is somewhat variable, arising by modification of the simple and compound meristogenous methods.

Spores are exceedingly variable in size and septation. Under some conditions nearly all spores are very small and unicellular with bicellular ones occurring rarely, while under other conditions the majority are quadricellular.

The principal agent of spore dissemination in the field is water during rains.

The fungus gains entrance directly through the cuticle after forming an appressorium at the tip of the germ tube. An intercellular mycelium is formed.

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OVERWINTERING OF PHYTOMONAS PRUNI ON PEACH

H. H. THORNBERRY[1] AND H. W. ANDERSON

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The overwintering of *Phytomonas pruni* on peach has been reported by Rolfs (6), Roberts (5), Adams (1), Higgins (4), and Dunegan (3) to occur in twig cankers. Rolfs (6) also mentioned that in some cases bacteria in infected bud tissue were the source of the spring inoculum. Anderson (2) presented evidence that the pathogen can survive the winter in diseased fallen leaves. Since he obtained negative results in his isolation from buds and from overwintering twig cankers he considered old fallen leaves as a possible source of inoculum in the spring. On March 22, 1930, a new type of twig canker was discovered at Olney, Illinois. This type will be referred to below as "spring canker" to distinguish it from the ordinary canker formed during the summer months.

DESCRIPTION OF SPRING CANKERS

The spring cankers, developing on young succulent twigs of the past summer's growth, form water-soaked, slightly darkened blisters about the time the first leaves appear. At first they are small, raised areas about 2 mm. in diameter, but later extend from 1 to 10 cm. parallel to the long axis of the twig (Fig. 1, A). They seldom extend more than one-half the circumference of the twig but may surround it. The bacteria are limited to the cortical tissue (Fig. 2, A). Occasionally, in cankers near the terminal bud, the infection surrounds the twig and a type of die-back results (Fig. 3). Later in the spring the epidermis over the lesion is ruptured (Fig. 4) and bacteria are exposed for dissemination by rain and dew to near-by leaves. After the epidermis is ruptured the infected tissue, darkening and drying during summer, results in deep cankers (Fig. 1, B), which persist through the following winter. Occasionally, several small separated blisters may coalesce to form a single large canker. They develop before green shoots are formed in the spring. Under the microscope the infected tissue is seen to be invaded intracellularly (Fig. 2, C) by abundance of bacteria. These ooze from the cut surface of sections mounted in water.

SUMMER CANKERS

The summer cankers on green shoots (Fig. 5) develop later in the summer, usually after foliage infection has become established. In 1932, for example, they did not appear until mid-August. At first they are water-

[1] Formerly assistant in Pomological Pathology, Ill. Agr. Exp. Sta. Now with Rockefeller Institute for Medical Research, Princeton, N. J.

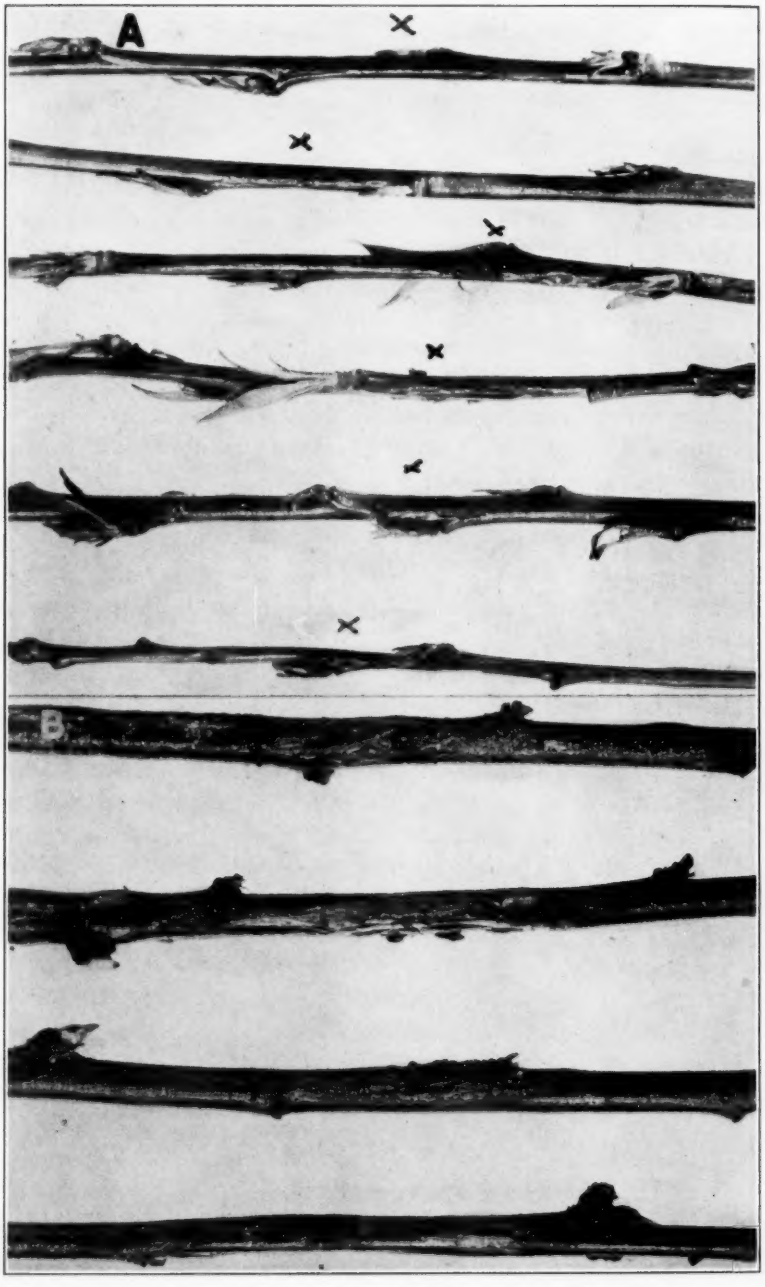

FIG. 1. Spring cankers on twigs and water sprouts. A. Young specimens collected at Villa Ridge on April 1, 1930. B. Note splitting and healing of overwintered specimens collected at Carbondale and Villa Ridge on May 20, 1930.

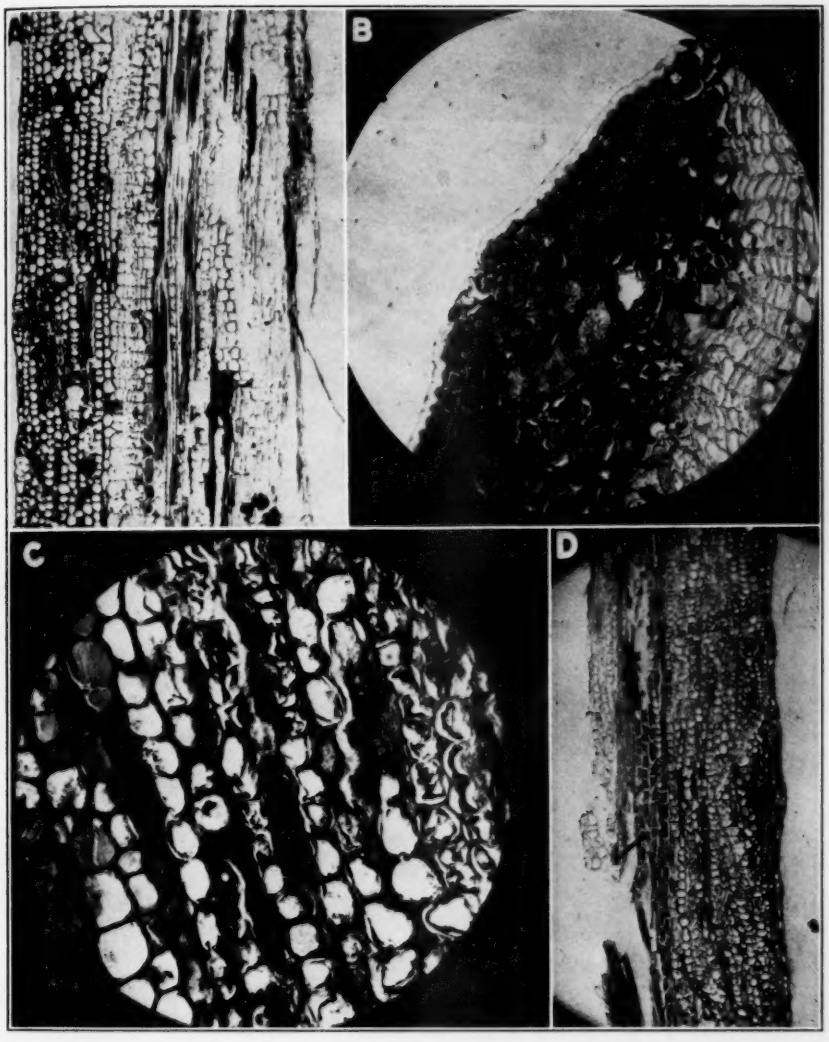

FIG. 2. Photomicrographs of stained sections. A. Masses of bacteria in the intercellular spaces of the cortical tissue of a young twig. Dark areas are bacteria. ×90. B. Bacteria in tissue. ×740. C. Same as A. ×740. D. Same as B. ×90.

soaked, dark purplish lesions that later dry out and show the characteristic sunken canker of darkened tissue.

PRIMARY FOLIAGE INFECTION

Natural, primary foliage infections in Illinois were correlated with the spring cankers during the early springs of 1930 and 1931 by the writers

FIG. 3. Primary foliage infection near a spring canker that caused a die-back of the twig. Collected at Urbana on May 12, 1931.

and in 1932 by the junior writer. Due to the scarcity of cankers in general and also the difficulty in locating them, it was much easier to find the twig lesions after primary infection was evident. However, many cankers were found before there was any evidence of foliage infection.

FIG. 4. Primary foliage infection, shown as dark areas on leaves, located below a spring canker at the base of the third shoot from the bottom. Canker has burst open. Collected at Villa Ridge on April 14, 1930.

In 1930, cankers were first observed in the University of Illinois peach orchard at Olney, Illinois, on March 22. At this time the early leaf buds had opened and no evidence of foliage infection was found. On April 1, at Carbondale, Villa Ridge, and at Irvington, cankers were found, but foliage infections were not evident near the cankers or elsewhere in the

orchard. On April 13, at Irvington, 7 leaves with very small spots that were quite typical of young lesions were found near a canker, while no infection could be found elsewhere in this orchard. Microscopical examination in the field showed bacteria oozing from the cut surface of one lesion. At Villa Ridge, on the 14th, light localized infections were found near cankers but none was noted in any other part of the trees. At Carbondale, however, on the same day, several cankers were observed but no foliage infection was found. At the time of the next observations, April 25, at Irvington and Carbondale, and April 26, at Villa Ridge and Olney, primary leaf infections were quite evident but only near cankers. The bacteria evidently had spread about one foot from the cankers. By the time of the next observations, May 20 and 21, the infection appearing throughout the trees prohibited further correlation of initial infection with spring cankers.

During the winter months of 1930 and 1931 the writers examined twigs at Urbana and in southern Illinois in order to determine when the cankers become evident. The study was carried out in orchards where the disease was prevalent the previous summer. On December 18 a careful search at Irvington, Carbondale, and Olney revealed no evidence of visible blistery cankers. However, the pathogen was isolated from a dead terminal bud collected at Olney. Again, at the same places on January 28, no cankers were found. On March 13 no cankers were observed either at Irvington or at Villa Ridge. On the 14th, at Carbondale, one small spring canker about 5 mm. in diameter was found on the south side of a water sprout. Isolation and microscopic examination the following day showed the pathogen throughout the lesion. On the 17th, at Carbondale, and 21st, at Irvington, a few cankers were observed without any foliage infection. However, on April 22, at Carbondale, 23, at Ozark, and 24, at Villa Ridge, primary infections were found near cankers. By May 10 the infections had appeared beyond the localized stage centered about cankers.

OBSERVATIONS ON PRIMARY INFECTION AT IRVINGTON, ILLINOIS, 1931–1932

An Elberta peach orchard near Irvington, Illinois, has yielded interesting results on overwintering, as it has always exhibited an abundance of bacterial spot. During the winter of 1931, a number of twigs showing typical summer cankers were collected and isolations attempted, with uniformly negative results.

On March 26, this orchard was examined for spring cankers but none could be found. Late in April primary infection was observed on a few leaves and, on May 24 and 25, a survey was made. From 85 trees, 70 typical spring cankers were obtained, mostly by examining the twigs near

cases of primary infection. Of these 70 cankers, 66 showed bacteria oozing from the cut outer bark when examined under the microscope, while 4 were negative. In addition, 53 tip-injured twigs that looked suspicious or were near primary infections were examined. Of these, 16 showed oozing bacteria, while 37 were negative. Those giving positive results were in most cases definite spring cankers at the base of the dead tip.

In addition to these observations, numerous summer cankers, formed in 1931, were examined for primary infection in the neighborhood and for oozing bacteria. The results were entirely negative.

EXPERIMENTS WITH PRUNED TREES

Further evidence that the source of primary inoculum may be traced to spring cankers was gathered at Urbana by a pruning experiment on 6 Brackett peach trees. These trees had had severe bacterial spot over a period of 4 years. Hundreds of summer cankers were marked during the season of 1931 for a study of the time of development of these cankers and the relation of time of development to possible survival of the bacteria. Plates were poured from a total of 221 summer cankers from this variety in January, February, and March, 1932. None of these yielded *Phytomonas pruni*, a fact that agrees with results of numerous other attempts.

The 6 Brackett trees were somewhat isolated from the remainder of the trees in the peach orchard and the closest susceptible variety was 6 rows distant.

Two trees were so pruned as to remove all 2- and some 3-year-old wood. All of the 1931 twigs were removed from the 2 trees, while the other 2 were left intact. It was reasoned that if primary infection appeared only on the nonpruned trees at the time of the first evidence of infection in the orchard, it also could be reasoned that the source of the inoculum was from year-old twigs. It was realized that secondary infections would soon appear on the pruned trees, but the time and place of primary infection, whether or not associated with spring cankers, would give a clue as to the place of overwintering of the bacteria.

Primary infection in the University peach orchards at Urbana was unusually late in the summer of 1932. The first observed infection was on a Hale tree on June 2. The nonpruned Brackett trees showed a light leaf infection a few days later, with no infection on the pruned trees. Since the pruned trees came into leaf a few days later than the nonpruned ones and it was thought this might be a factor in primary infection, 10 days were allowed to elapse before records were taken. On June 12, a number of infection areas were observed in the nonpruned trees where the leaves showed a few spots. In most instances the source of the inoculum was

evidently from typical spring cankers. Neither of the pruned trees that had 2 years' growth removed showed infection. One of the trees having one-year twigs removed also was free of infection. The second had one area where the leaves on a single small branch were infected. An examination of this branch revealed a canker immediately below the point where a twig had been removed.

For 3 weeks following no diseased leaves were found on the 3 trees, while extensive secondary infection occurred on the nonpruned trees and the infection area on the one pruned tree increased in extent. During the latter part of August, diseased leaves were found on all trees.

It would appear from this limited experiment that the primary inoculum comes from cankers on year-old wood, and that the spring cankers rather than the typical summer type of canker constitute the probable source.

ISOLATIONS FROM CANKERS

Isolations from the spring cankers soon after they were found showed abundance of typical colonies of *Phytomonas pruni* in the plates. From a total of 530 plates, 265 isolations duplicated, all contained the pathogen (Table 1).

Similar isolations from cankers throughout the following winter and spring indicated that there was a great reduction of the number of bacteria

TABLE 1.—*Summary of isolation results secured from various specimens of spring cankers of peach*

Collection		Isolation			
Date	Locality	Date	Specimens	No. Plates	Results
3/22/30	Olney	3/24/30	20	40	40+
3/22/30	Olney	3/27/30	15	30	30+
3/22/30	Olney	4/3/30	20	40	40+
4/1/30	Villa Ridge	4/3/30	10	20	20+
4/1/30	Carbondale	4/3/30	30	60	60+
4/1/30	Irvington	4/3/30	40	80	80+
4/1/30	Carbondale	4/7/30	10	20	20+
4/2/30	Olney	4/3/30	10	20	20+
4/25/30	Carbondale	4/27/30	30	60	60+
4/25/30	Irvington	4/27/30	20	40	40+
4/25/30	Villa Ridge	4/27/30	30	60	60+
6/17/30	Dixon, Ky.	6/20/30	10	20	20+
7/2/30	Urbana	7/2/30	10	20	20+
3/14/31	Carbondale	3/16/31	10	20	20+
			265	530	530+

in the tissue. From a total of 370 plates, 185 isolations duplicated, one showed typical colonies of the pathogen. The organism from a subculture of a colony proved to be pathogenic when inoculated by pricks with a needle into green Elberta shoots on June 6, 1931 (Table 2).

TABLE 2.—*Summary of isolation results from overwintering summer cankers*

Collection		Isolation			
Date	Locality	Date	Specimens	No. Plates	Results
12/19/31	Irvington	12/20/31	15	30	30 –
1/28/31	Irvington	1/30/31	20	40	40 –
1/28/31	Carbondale	1/30/31	20	40	40 –
1/28/31	Tunnel Hill	1/30/31	25	50	50 –
1/28/31	Irvington	2/4/31	10	20	20 –
1/28/31	Carbondale	2/4/31	20	40	40 –
1/28/31	Tunnel Hill	2/4/31	10	20	20 –
3/14/31	Carbondale	3/16/31	5	10	1 +
3/14/31	Villa Ridge	3/16/31	10	20	20 –
3/14/31	Irvington	3/16/31	15	30	30 –
3/14/31	Carbondale	3/20/31	10	20	20 –
3/14/31	Irvington	3/20/31	10	20	20 –
3/14/31	Villa Ridge	3/20/31	15	30	30 –
			185	370	1 +

Diseased tissue from the cankers that develop on green shoots from natural infection during spring and summer (Fig. 5) also was used for plating. The cankers were located and labeled on July 10, 1929, at a time when their macroscopic identification was reasonably certain. Isolation from 5 on that date proved that bacteria were present in abundance. On January 5, 18, 24, 29, February 7, 13, 25, and 28, 1930, all the isolations were negative. Similar studies on the same dates with cankers produced by artificial inoculation at Urbana likewise developed no typical colonies of the pathogen. The inoculations with culture No. 66 were made into young green Elberta shoots by pricking with a needle on July 15, 1930. Typical lesions containing abundance of bacteria were evident July 25. These results agree with those of the junior writer, extending over a period of 10 years and involving approximately 500 attempts at isolation from summer cankers during the winter months. In not a single case was *P. pruni* isolated from such cankers after December 1.

OVERWINTERING OF THE PATHOGEN IN CULTURE

The pathogen in culture, protected from direct sunlight and rain, survived the outdoor winter conditions of 1930–31 at Urbana. Dextrose-beef-

extract agar slants, freshly inoculated with a culture of *Phytomonas pruni* were repeatedly exposed about the 1st and 15th of each month from November to March, inclusive. Transfers from the cultures to sterile slants on April 1 showed viable bacteria in all on April 5. There was noticeable growth in the exposed tubes during periods when the mean maximum temperature exceeded 55° F. or 13° C. for 3 days or longer. (The Illinois

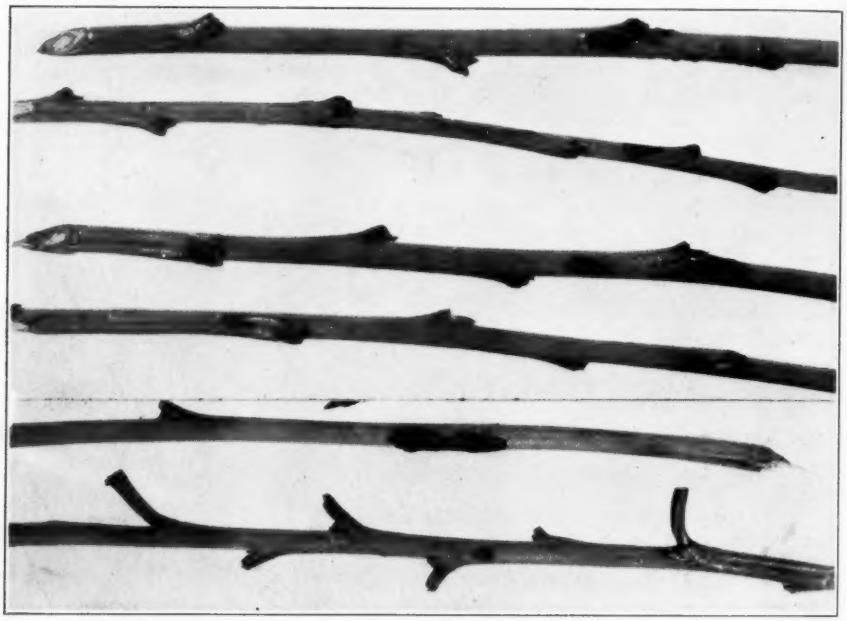

FIG. 5. Cankers produced from natural field infection on green shoots during the summer. Collected November 27, 1930.

section of The Climatological Data was the source of temperature records). In cultures kept in a refrigerator at $-2°$ to $+2°$ C. the organism was viable after 5 months. The optimum, minimum, and maximum temperatures for growth of the bacteria were found to be 25°, 7°, and 38° C., respectively. These were determined from 10 freshly inoculated dextrose-agar slants incubated at temperatures of 4°, 7°, 12°, 18°, 25°, 32°, 38°, 46°, and 52° C., for 10 days. No growth was evident outside the range of 7° to 38° C. The organism that had been held at 4° C. grew when incubated for 3 days at 25° C., while those at 46° and 52° C. were not viable. Thermograph records showed that the temperature varied less than 2° C. throughout the experiment. The thermal death point was found to be 52° C.

OVERWINTERING IN TWIGS

The pathogen survived the winter of 1931–32 at Yonkers, New York,[2] in peach twigs artificially inoculated at intervals of about 2 weeks, from November to June, inclusive. Needle-prick inoculations, consisting of 100 punctures into the cortical tissue of young twigs with culture No. 202, were made on November 9, 14, December 3, 15, 1931, January 1, 15, February 3, 15, March 1, 17, April 3, 15, May 2, 14, June 2, and 19, 1932. One hundred pricks with a sterile needle on each of these dates served as controls. On February 3, 2 of the inoculations of November 9 near the tip of a twig had developed typical blistery cankers about 5 mm. in diameter around the puncture. On April 15, 10 additional cankers appeared from the inoculation of November 9 and one from the February 15 inoculation. By May 22, one other canker appeared from the February 15 inoculation. At the last observation, August 18, no other cankers had developed. Thus, from a total of 1,600 inoculations, 14, or .875 per cent, developed typical spring cankers. However, 12 per cent of the inoculations of November 9 developed cankers. All the infections artificially produced were those near the tip of the twigs. In no case did infections result from the inoculations into 2-year-old twigs.

Primary leaf infections near the cankers were observed on May 22 when no infection was found elsewhere in the orchard.

Isolation plates from the leaf spots found May 22 and from the cankers on February 3, April 15, May 22, and August 18, all developed typical colonies of *Phytomonas pruni.* The last isolation, however, showed that the bacteria in the diseased tissue had decreased considerably in number during the summer. Stained microtome sections of the cankers collected and fixed on February 3 and April 15 contained bacteria in the diseased tissue.

ISOLATIONS FROM OVERWINTERING LEAVES

Since the observations made by Anderson (2) indicated that overwintering on fallen leaves was possibly the source of the primary inoculum, some additional attempts have been made to obtain positive evidence on this point. During the winter and spring of 1931–32 isolations of *Phytomonas pruni,* made from fallen leaves as late as February 8, indicate that the bacteria may survive in fragments of fallen leaves throughout the winter months. No direct evidence that the primary inoculum comes from this source, however, has been adduced.

[2] The results of this experiment are included in this paper with the permission of the Director of The Boyce Thompson Institute for Plant Research, Yonkers, New York.

DISCUSSION

The spring cankers differ from the summer twig cankers in the time of their development, appearance, and infection. The former develop on the tip portion of overwintering twigs and on water sprouts before green shoots are produced, while the latter develop only in the green tissue of the shoot. Inoculations of overwintering twigs throughout the spring until June 15, at the time when green shoots were susceptible, indicated that they were resistant. The twig inoculations during the fall and winter of 1931–32 showed that the pathogen, if introduced by puncture, can survive the winter in the twigs and produce typical cankers the following spring. The summer cankers develop from infections usually after foliage infection is well established. Both types of cankers contain abundance of bacteria when they are first formed, but during the summer they dry considerably and there is a marked diminution of bacteria. Throughout the following winter and spring it is difficult to isolate the pathogen from either type of canker. This difficulty has already been mentioned by Anderson (2) and Dunegan (3). The overwintering cankers of either type are not considered to be the principal source of inoculum for primary foliage infection. The cankers shown by Dunegan (3) in figure 6, B, appear quite similar to those in figure 5, which are known to develop early in the spring. Only the early stage of the spring cankers supply this inoculum.

Why *Phytomonas pruni* does not survive the summer in twig cankers may be explained from the conditions found in stained microtome sections. Very shortly after inoculation the host reacts by walling off the infected area by the formation of corky layers of cells, distinctly visible in all sections of cankered twigs (Fig. 2, B and D). If invasion occurs outside this layer, due to extreme activity of the bacteria, a second layer of cells forms (Fig. 6, A), and this process is repeated until the bacterial areas are successfully isolated. Any canker visible to the naked eye during the growing season is thus effectively separated from the active cortical tissue.

As the growth of the host tissue ceases in the late autumn any invasions resulting from late infections are not so isolated and may continue to develop additional bacterial cells and remain in intimate contact with the cortical cells. In the early spring before the host cells start division, the masses of bacteria invade the cortical region by the formation of zoogloeal masses, as described by Nixon and others in the case of *Erwinia amylovora*.

The older basal portion of the twig is more resistant to invasion than is the apical area. This probably is due in part to the fact that the stomata are older and less active and, in part, to the presence in the older portion of a phellem layer beneath the epidermis, which walls off the cortex against external invasion. The resistance of the overwintered twigs to invasion,

even when the cortex is punctured, may be explained by the compact and relatively inactive character of the cortical cells. Cankers have not been observed to develop on 2-year-old twigs. Also, infections failed to develop from inoculations into these twigs.

The incubation period for twig infections was found by Rolfs (6) to be from 4 to 10 days. Dunegan (3) found that it varied from 7 to 12 days and that cool weather prolonged the incubation period in leaf infections to 25 days. In twig inoculations throughout the past winter the period was prolonged to 86 and 157 days in the November 9 inoculations and from 59 to 96 days in the February 15 inoculations. The pathogen in culture remained viable when exposed to outdoor winter conditions and to ice-box conditions of $-2°$ to $+2°$ C. for 5 months. In a typical leaf lesion

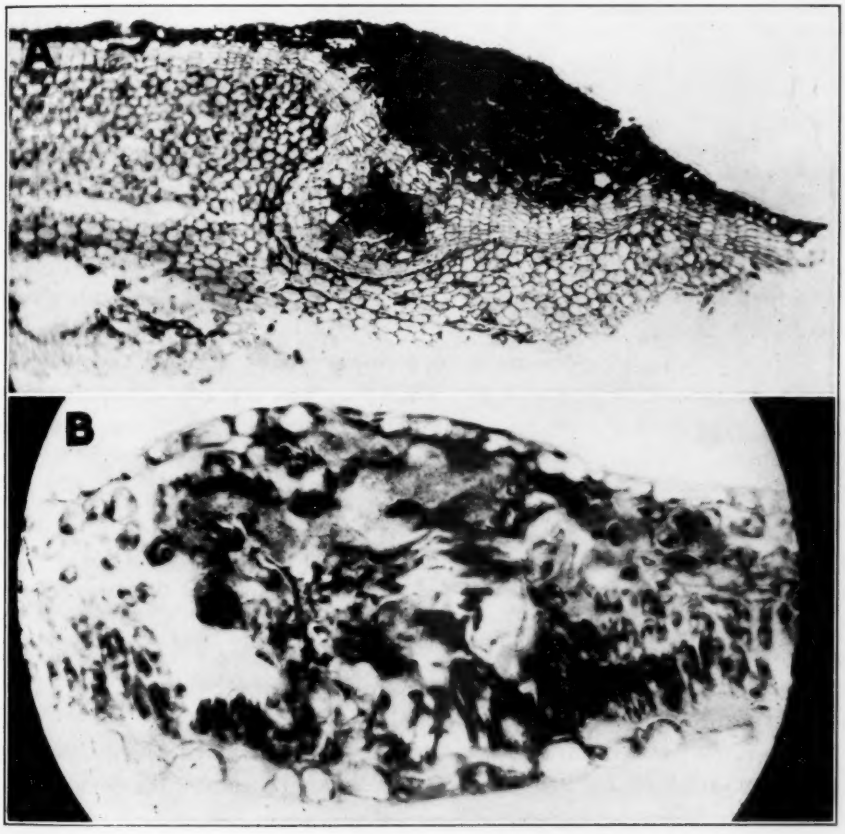

FIG. 6. Photomicrographs of stained sections. A. Secondary invasion, walled off by new layers of cambium. ×90. B. Bacteria in an Elberta leaf two months after inoculation in the greenhouse. ×740.

that developed in the greenhouse 2 months after inoculation, bacteria were visible in stained microtome sections. (Fig. 6, B.) During the incubation period was prolonged to 86 and 157 days in the November 9 inoculations rather low.

The temperature throughout the winter and spring appears to determine the time of development of spring cankers into visible lesions. Their appearance in southern Illinois seemed to be correlated with a mean maximum temperature of about 12° C. (53.6° F.) or higher for 5 or 6 days.

The time of twig infection is probably during rains before the infected leaves drop in the fall. After the organism has become established in the cortical tissue temperature seems to be the controlling factor for determining the incubation period. Noticeable lesions may not develop for 2 or more months after the initial infection, as was found in artificial inoculations into young twigs. It is believed that infections that become noticeable lesions or produce appreciable injury to the twigs before the growth is retarded by low temperature are corked off and are dried during the winter. Only late infections that occur shortly before the rest period of the host tissue starts constitute the source of canker bacteria the following spring.

SUMMARY

Spring cankers, a new type of twig lesions, were found in 1929. They develop on the previous summer's growth and on water sprouts shortly after the first leaf buds open.

Primary foliage infections have been correlated for 3 years with the spring cankers. Trees pruned of their 1-year and 2-year twigs, thus eliminating spring cankers, were not infected until bacterial spot was general in the orchard.

The pathogen was repeatedly isolated in 530 plates from young spring cankers. From overwintering cankers only one plate out of 370 developed *Phytomonas pruni* colonies. Isolations from young summer cankers were all positive, while those from overwintering cankers were negative.

The pathogen in culture survived the winter conditions of 1930–31 at Urbana and ice-box conditions of − 2° to + 2° C. for 5 months. Its optimum, minimum, and maximum temperatures for growth are 25°, 7°, and 38° C., respectively. Its thermal death point is 52° C.

The organism in artificial twig inoculations survived the winter conditions of 1931–32 at Yonkers, New York, and produced typical spring cankers the following spring.

DEPARTMENT OF HORTICULTURE,
AGR. EXPT. STA.
URBANA, ILLINOIS

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CONTROL OF GLADIOLUS SCAB

L. E. MILES

(Accepted for publication Jan. 6, 1933)

Studies on the control of gladiolus scab caused by *Bacterium marginatum* were started in the spring of 1927 by Mr. H. H. Wedgworth, at that time Associate Plant Pathologist for the State Plant Board of Mississippi. The results secured by him in 1927 and 1928 were published in November, 1928, in Mississippi Bulletin No. 261, entitled Investigations on Diseases of Vegetable and Ornamental Plants. The writer took up the project in the fall of 1928 at the point where it was left by this publication. Four additional years of work have been devoted to it, so that it is now felt that fairly conclusive statements can be made relative to the control of this, the most prevalent disease of the gladiolus.

When this project was undertaken by Mr. Wedgworth, and, likewise when it was taken up by the writer, no work on a sufficiently comprehensive basis had been done with respect to it, so that, in consequence, there was great diversity of opinion as to the value of disinfection of gladiolus planting stock. When disinfection was practiced or attempted, the methods and materials used were even more varied and various. Treatments reported to the author as having been used with differing degrees of success have ranged all the way from coal soot through sulphur, the organic mercury dusts to liquid treatments such as Semesan, bichloride of mercury, and even to soap suds.

It was therefore considered desirable to secure some definite information as to the relative efficiency of at least some of the most promising from this multiplicity of materials and methods. With that end in view, the work begun by Mr. Wedgworth was continued and on a more comprehensive basis.

SOURCE OF MATERIALS

Letters explanatory of our plans and purposes were addressed to gladiolus growers in all sections of the country requesting their cooperation by donation of both diseased and healthy corms. A very hearty response was received to these requests and a large number of corms representing all degrees of infection was secured. The average condition of the diseased corms as used in the following tests with respect especially to the infection present is shown in figure 1. Many varieties were represented, but fortunately a large enough number of a single variety was usually available to make up the more important test plats. The author wishes here to express his thanks to all growers who rendered such invaluable assistance.

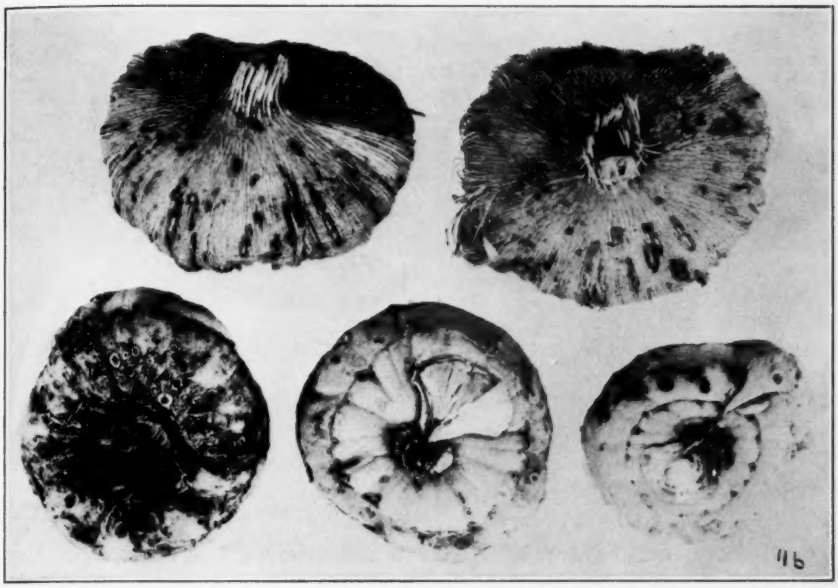

FIG. 1. Average conditions of corms planted in these tests as moderately or severely infected with scab.

Materials used in the treatment of corms and soil were in most cases contributed by the manufacturers. Thanks also are due to these companies for their generous cooperation.

EXPERIMENTS ON CONTROL OF GLADIOLUS SCAB BY CORM TREATMENT

In 1929 a test was designed to show the relative efficiency of various lengths of treatment with corrosive sublimate, Semesan, and formaldehyde. A single variety, America, was used in this test. The lengths of time of treatment for each of the disinfectants were 6, 8, 12, and 16 hours. The number of corms in each treatment was 125, and the test was repeated once, making a total of 250 corms per treatment. The corms were treated on March 18, 1929, and planted on the following day. Table 1 gives a summary of the results secured.

Examination of table 1 reveals that most efficient control of scab was secured from the 12-hour soak in mecuric chloride, followed next in order by the 8-hour soak, with the 16-hour treatment period only shortly behind it. The 6-hour period apparently was too short to give efficient results with this material. On the other hand, with Semesan, the 6-hour treatment gave the largest number of clean corms of any time period used. It was, however, below all treatments with bichloride of mercury except the 6-hour period.

TABLE 1.—*Effect of length of treatment with $HgCl_2$, Semesan, and formaldehyde, 1929*

Treatment	Length of time	Germination	Increase	Clean	Scabbed
	Hrs.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
$HgCl_2$ 1–1000	6	96.0	92.4	57.7	42.3
Semesan 1–400	6	92.8	101.4	84.1	15.9
Formaldehyde 1–240	6	92.0	27.6	32.0	68.0
$HgCl_2$ 1–1000	8	97.5	102.6	91.8	8.2
Semesan 1–400	8	90.4	106.0	77.7	22.3
Formaldehyde 1–240	8	100.0	23.0	64.9	35.1
$HgCl_2$ 1–1000	12	95.2	111.2	97.5	2.5
Semesan 1–400	12	88.8	127.6	83.3	16.7
Formaldehyde 1–240	12	94.9	33.2	28.1	71.9
$HgCl_2$ 1–1000	16	92.8	64.0	89.8	10.2
Semesan 1–400	16	96.0	100.7	76.0	24.0
Formaldehyde 1–240	16	92.3	25.4	41.7	58.3
Check	.....	96.4	48.7	6.5	93.5

Formaldehyde ranked far behind both these in the percentage of clean corms and was decidedly inefficient.

When the percentage increase in crop is considered, it is found that it follows in the main the percentage of clean corms harvested. Some variations do occur, as, for instance, the fact that the largest increase in crop is produced by the 12-hour treatment with Semesan, while that treatment ranks fifth in the percentage of clean corms produced. Formaldehyde produced even a smaller increase in crop than did the untreated lots, although the percentage of clean corms was very decidedly larger. Results shown in this table confirm the conclusion reached by Wedgworth that formaldehyde should not be recommended for the treatment of gladiolus corms against scab.

In 1930 an additional supply of corms was secured from growers and a comprehensive lot of tests designed and planted. A very severe and prolonged drought occurred, however, and affected the plats so severely that no results were secured. The dry period began just as the plants were beginning to bud and affected them to such an extent that many died in the field without blooming. Others bloomed but later died without making a new corm. Such corms as were harvested were almost entirely free of scab, regardless of the treatment received by the particular plat from which they came. It was evident, therefore, that the unusual weather conditions had held the disease in check to such an extent that all treatment results were vitiated and rendered worthless.

The 1930 series of tests were, therefore, in large measure, repeated in 1931 (Fig. 2). The 1930 crop of corms, being largely disease free, as a

FIG. 2. A partial view of the 1931 gladiolus-scab-control test plots, giving some idea of their extent.

result of the weather conditions referred to above was carefully sorted and used chiefly for checks, and new diseased materials were secured from the growers who had cooperated in previous years. In table 2 are presented the results of a test of the efficiency of bichloride of mercury, and the substances Calogreen and Semesan. Calogreen is a mercurous mercury compound, being in fact, a form of calomel. The corms were treated and planted on March 11 and 12, 1931. The variety, Ida Van, was used. Each treatment consisted of 100 corms and the treatments were replicated 4 times, making a total of 2,400 corms in all in the test.

This test shows Calogreen standing far in advance of any other material in its efficiency in scab control. Commercially, the corms harvested from

TABLE 2.—*Gladiolus corm treatments with $HgCl_2$, Calogreen and Semesan, 1931*

Treatment	Concentration	Time	Increase	Clean	Scabbed
			Per cent	*Per cent*	*Per cent*
Check (diseased)	.....	.....	28.5	6.4	93.6
$HgCl_2$	1–1000	8 hrs.	111.4	87.2	12.8
Calogreen	1 lb.–10 l.	5 min.	190.0	98.1	1.9
Semesan	1 oz.–12 l.	5 min.	108.9	78.8	21.2
Check (clean)	.....	.....	141.7	96.3	3.7

the plat treated with this material would have been considered 100 per cent clean and the increase in the weight of crop harvested was far beyond any other treatment, although the clean check gave an abundant harvest. Bichloride of mercury and Semesan ranked very much the same as they had done in previous tests but both were much less efficient than was Calogreen.

Another test was planned to try out the efficiency of Calogreen at different concentrations. The rate previously tested, 1 pound to 10 liters of water, was used again, and with it was compared a concentration only one-half as great, namely, ½ pound to 10 liters of water. In this test was used also an organic mercury product designated as 80–A, provided for the purpose by the Bayer-Semesan Company. It was used at the rate of 1 pound to 12 liters of water. Calogreen and 80–A formed thick suspensions and the corms were allowed to remain in them for a period of 5 minutes. Frequent agitation was practiced to keep the materials thoroughly in suspension and to assure that all the corms were completely in contact with the chemicals. All of these short soaks were again checked against a soak of 8 hours in a 1–1000 solution of bichloride of mercury, which was used throughout all of these tests as a standard for comparison. Diseased corms only were used as a check. One hundred corms of the variety Le Marechal Foch were used for each treatment. The treatments were quadruplicated, thereby making a total of 2,000 corms in the entire test. A summary of the results secured is presented in table 3.

TABLE 3.—*Summary of short-soak treatments compared with 8-hour soak in 1–1000 $HgCl_2$, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check (diseased)	.....	.....	0.0	91.1	13.2
$HgCl_2$	1–1000	8 hrs.	80.8	3.4	40.8
Calogreen	1 lb.–10 l.	5 min.	96.8	0.0	71.0
Calogreen	0.5 lb.–10 l.	5 min.	90.5	0.9	63.2
80–A	1 lb.–12 l.	5 min.	61.2	22.7	23.8

Both concentrations of Calogreen gave efficient control. The stronger concentration, however, gave a higher percentage of clean corms and also a greater number averaging No. 1 in size. The product, 80–A, did not give efficient control of scab at the concentration used. This test agrees with the one previously reported in placing Calogreen first in rank in efficiency of all materials tested.

The next test to be reported consisted of a comparison of 2 treatments with a substance sold under the trade name Calochlor, using again an 8-hour soak in a 1–1000 solution of bichloride of mercury as a standard for comparison. Calochlor is reported to be a mixture of mecurous and mecuric chlorides. It was used at a concentration of 3 ounces to 5 gallons of water. One lot of corms was soaked for 5 minutes and another for 30 minutes. Almost constant agitation was necessary to keep the Calochlor in suspension. The variety Alice Tiplady was used in this test, at the previous rate of 100 corms per treatment. The treatments were again quadruplicated, making a total of 1600 corms in this test. The results secured are summarized in table 4.

TABLE 4.—*Comparison of 8-hour soak with $HgCl_2$, 1–1000, with Calochlor, 5-minute dip and 30-minute soak, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check			2.1	93.8	14.1
$HgCl_2$	1–1000	8 hrs.	91.1	1.2	83.0
Calochlor	3 oz.–5 gals.	5 min.	62.0	19.2	44.1
Calochlor	3 oz.–5 gals.	30 min.	69.9	17.7	33.8

Neither of the Calochlor treatments shows in this test a sufficiently high degree of efficiency to warrant its further use. Germination was delayed and the flower spikes were shorter than in any other plot in the entire series of tests. This was especially marked in the 30-minute-soak treatment.

Reports from various sections of the country have indicated that a short soak with a solution of Gold Dust has sometimes given effective control of scab. Others have reported that a 2-hour soak in a solution of acetic acid has been satisfactory. It was, therefore, thought advisable to test these 2 methods against the 8-hour soak in 1–1000 bichloride solution. The Gold Dust was used at the rate of 6 ounces to 10 liters of water and the time period set at 45 minutes, which was a compromise between the 30-minute and 1-hour periods seemingly in most frequent use. Acetic acid of 56 per cent concentration was used at the rate of 8 pounds to 50 gallons of water for a period of 2 hours. 100 corms of a single variety, Evelyn Kirtland, were used in each treatment, and the test was triplicated, making a total of 1200 corms in all. The summary of results secured is presented in table 5.

Examination of the table will show that both acetic acid and Gold Dust proved decidedly inefficient in this test, the harvested crop of corms being

TABLE 5.—*8-hour soak in 1–1000 $HgCl_2$, 2-hour soak in acetic acid, and 45-minute soak in Gold Dust, 1931*

Treatment	Concentration	Time	Clean	Severe scab	No. 1
			Per cent	*Per cent*	*Per cent*
Check (diseased)			0.0	98.1	7.6
$HgCl_2$	1–1000	8 hrs.	79.2	7.2	68.0
Acetic acid	8 lbs. 56%–50 gals.	2 hrs.	3.9	89.7	17.9
Gold Dust	6 oz.–12 l.	45 min.	1.4	92.3	22.8

in but little better condition than that from the nontreated diseased check plat. The Evelyn Kirkland corms used in this test were small, mostly numbers 2 and 3, and were very severely scabbed. Even at that, however, the 8-hour soak with 1–1000 bichloride gave a fairly clean crop.

Having available a considerable quantity of corms in relatively small number, that is, varying from 100 to 500 corms per variety, a test was designed to determine whether there was a possible difference in response to a particular treatment between different varieties. Seven varieties were divided into lots of 100 corms each, the number of such lots varying with the number of corms of the variety available. Each lot of 100 corms was again divided into 2 lots of 50 corms each. One of these was not treated, and the other was treated for 5 minutes in a solution of Calochlor, made up at the rate of 3 ounces to 20 liters of water.

TABLE 6.—*Varietal response to 5-minute soak in Calochlor, 3 ounces to 20 liters, 1931*

Variety	Treatment	Number of lots	Clean	Severe scab
			Per cent	*Per cent*
Le Marechal Foch	Check	1	3.6	88.9
" " "	Treated	1	70.0	13.9
Los Angeles	Check	8	1.1	93.9
" "	Treated	8	59.8	19.9
Merton W. Wentworth	Check	1	0.0	100.0
" " "	Treated	1	48.2	26.1
Mildred Adeline	Check	1	8.1	79.0
" "	Treated	1	74.4	6.8
Mrs. Dr. Norton	Check	1	2.9	87.8
" " "	Treated	1	60.7	20.1
Myra	Check	6	3.1	79.9
"	Treated	6	66.2	17.6
Myrtle	Check	1	1.2	91.0
"	Treated	1	68.4	16.7

In this case the choice of the disinfectant proved an unfortunate one. Calochlor, in harmony with the data recorded in table 4, gave unsatisfactory results as a disinfectant for the control of gladiolus scab. It would seem, however, from this test that there is no marked difference in the response of different varieties to the action of the disinfectant. As in a previous case where Calochlor was used, all varieties showed delayed germination and a stunted condition of the plant, which was never overcome. When the corms of the Merton W. Wentworth variety were examined after harvest, it was found that a considerable number of them were affected with Fusarium rot, in addition to scab. The presence of this additional disease may be in part responsible for the low percentage of clean corms secured in the case of this variety.

A second test, similar to the one just recorded, included only 6 varieties. The treatment consisted of a 5-minute soak in a suspension of Calogreen made up at the rate of 1 pound to 10 liters of water. Results are recorded in table 7.

TABLE 7.—*Varietal response to 5-minute soak in Calogreen, 1 pound to 10 liters, 1931*

Variety	Treatment	Number of lots	Clean	Severe scab
			Per cent	*Per cent*
Myrtle	Check	1	2.0	92.1
"	Treated	1	97.3	0.0
Rose 1910	Check	2	0.0	87.3
" "	Treated	2	93.9	0.0
Odin	Check	3	6.5	81.6
"	Treated	3	99.2	0.0
Pink Beauty	Check	6	0.0	100.0
" "	Treated	6	92.0	0.0
Schwaben	Check	5	1.3	90.6
"	Treated	5	94.9	0.0
Souvenir	Check	2	10.1	69.3
"	Treated	2	98.3	0.0

In this case, again, Calogreen proved to be very efficient. The most severely infected corm in any of the lots treated with it would ordinarily be passed by the commercial grower as perfectly clean. No corm treated with Calogreen at the rate of 1 pound to 10 liters of water has been found in any of these tests that has been either moderately or severely infected with scab.

Taking into consideration the results secured in the last 2 tests recorded, it may be reliably concluded that there is no difference in the response of a particular variety to the action of the disinfectant used. A total of 13 varieties has been tested with no evidence of such response.

Having some land available on which diseased gladioli had been grown in previous years, some tests were designed to get some information regarding a possible effect of disinfectants added to the soil about the corms on the prevention of infection by the soil-borne organisms. The plots chosen were two in which diseased crops of corms were grown in 1928 and 1929, respectively. No gladioli were ever grown in either of them except the crop hereinbefore mentioned. Four substances were used for soil treatment, namely, Calochlor, Calogreen, Semesan, and lime. The latter was used because of the claim made by a grower that he had repeatedly grown a clean crop of corms on infested soil by adding lime to the soil in the row. Four 24 ft. rows constituted a single plot. One row between each 2 plots and one on the outside of the end plots, 5 rows in all, left untreated, constituted the check plot. The Calogreen, Calochlor, and Semesan were applied at the rate of 6 ounces per 1000 feet of row, or 21.8 grams per 24-foot row. The lime was applied at the rate of 2 tons per acre, or 2.2 pounds per 24 foot row. The disinfectants and lime were mixed with the soil immediately adjacent to and above the corms. A total of 25 varieties was used in this test, 50 corms of a single variety constituting one row. All corms were severely infested with scab and all were soaked for 8 hours in a 1–1000 solution of bichloride of mercury. Results of the tests are summarized in table 8.

TABLE 8.—*Treatments of infested soil. Scabby corms treated 8-hours in 1–1000 $HgCl_2$, 1931*

Treatment	Diseased crop in 1928		Diseased crop in 1929	
	Clean	Severe scab	Clean	Severe scab
	Per cent	*Per cent*	*Per cent*	*Per cent*
Calochlor	88.3	4.0	79.9	7.1
Calogreen	95.1	0.0	91.3	0.3
Lime	31.0	24.2	40.1	37.6
Semesan	63.2	11.6	58.9	15.2
No Soil Treatment	48.8	26.3	45.0	30.9

Examination of this table reveals that soil infestation from both the 1928 and 1929 crops is abundantly in evidence. The percentage of clean corms on the soil that received no treatment is much lower than that to be expected from an 8-hour soak in 1–1000 bichloride of mercury, when the corms are grown in clean soil. The percentage of severely scabbed corms is much higher than when similarly treated corms are grown in soil where gladioli had not been grown previously. There is also some evidence to show that soil infestation is somewhat greater on soil where the diseased crop was grown most recently.

Lime had no effect in controlling infection from soil-borne organisms. In fact, in both cases the percentage of clean corms was smaller on the limed plot than on the plots that received no soil treatment. The excess amount of lime present also retarded the growth of the plants and greatly reduced the yield. Calochlor and Semesan were apparently somewhat effective in reducing infection from the soil, although Calochlor was much the less efficient of the two. Calogreen would seem to have been extremely efficient in preventing such infection. The percentage of clean corms was as high as, or even higher than, would be expected to have resulted from the 8-hour soak in bichloride of mercury, even when the crop is grown on disease-free soil.

Another test was designed to determine the efficiency of the same materials in preventing infection from soil-organisms when clean corms were used. The soil chosen was that on which a diseased crop had been grown in 1929. In this test 100 corms constituted a row. The soil in 2 rows was treated with one of the materials and that of the next 2 rows was not treated and served as a check. The soil of the next 2 rows was treated with another of the materials, with the corresponding pair of rows as a check, and so on throughout the test plot. The materials were applied to the soil at the same rate as in the preceding test. The variety, America, was used for the Calogreen-treated plot and its corresponding check, and Chicago White, E. J. Shaylor, and Sunbeam for the lime, Calochlor and Semesan plots, respectively. The data secured are recorded in table 9.

TABLE 9.—*Treatments of infested soil, 1931. Corms clean, nontreated*

Treatment	Clean	Severe scab
	Per cent	*Per cent*
Calogreen	72.3	8.6
Lime	8.8	63.0
Calochlor	58.0	20.7
Semesan	22.1	40.7
Check	16.2	48.1

Examination of table 9 reveals the fact that a heavy soil infestation was present in the plots, as shown by the high percentage of severely scabbed corms harvested, even though apparently disease-free corms were planted. Again, the limed plot yielded more severely infected and fewer clean corms than did the nontreated, check plot. Both Semesan and Calochlor proved relatively inefficient in preventing infection from soil-borne organisms, when applied to the soil; and again, Calogreen showed its versatility as a disinfectant for use against gladiolus scab. It yielded by far the highest

percentage of clean corms of any treatment tested and likewise the smallest number of severely scabbed corms. It would seem, therefore, that Calogreen, applied to the soil immediately adjacent to the corm, might have an important place in scab control for the small grower whose available land is too limited to allow of proper rotation to avoid the use of infested soil.

EXPERIMENTS ON SPRAYING AND DUSTING FOR SCAB CONTROL

Since many growers have reported excellent results from the use of Bordeaux-mixture sprays and copper-lime dusts in the control of scab and other diseases of the gladiolus, it was considered advisable to test these methods of procedure. The writer used a 4–6–50 Bordeaux mixture, consisting of 4 pounds of copper sulphate, 6 pounds of hydrated lime, and 50 gallons of water. Since the gladiolus leaf is smooth and of such texture that spray does not readily stick to it, a spreader, composed of resin fish oil soap at a rate of 3 pounds to 50 gallons of spray, was added. The spreader gave the spray liquid excellent sticking and spreading qualities and was quite efficient. The dust applied was a home-made product consisting of 20 per cent monohydrated copper sulphate and 80 per cent of hydrated lime.

Three plots of 400 corms each of the variety Pink Beauty were used for this test. One plot was sprayed at intervals of 2 weeks with Bordeaux-mixture, beginning when the plants were 4 to 6 inches above the ground. Another plot was dusted with copper-lime dust on the same dates, and the third, a nontreated plot, served as a check. Six applications of both spray and dust were given, the last being shortly after the blooming period but before the plants had begun to turn yellow. The dust was applied in the early morning, while the plants were wet with dew. The variety Pink Beauty was chosen for this test because in previous years it had shown probably more foilage infection than had any other variety observed. The corms were all severely infected and averaged No. 2 in size. They were planted in soil in which gladioli had not been grown previously. A total of 200 corms in each plot was soaked for 8 hours in 1–1000 bichloride of mercury, and the remaining 200 were not treated. The results secured are presented in table 10.

Examination of table 10 shows that Bordeaux-mixture almost entirely eliminated foliage infection on plants from treated corms. When severely diseased corms were not treated prior to planting foliage infection was greatly reduced but not entirely eliminated. Even on the plants that did show foliage infection, the infection was much less severe than on the non-sprayed plants. The copper-lime dust was much less efficient than the Bordeaux-mixture spray. It is unfortunate that the test was not repeated on land known to be infested with the scab organism. This, however, was not done, since such land was not available at the time of the test. It is

TABLE 10.—*Spraying and dusting gladioli for scab control, 1931*

Treatment	Corms treated 8 hours in 1–1000 $HgCl_2$		Corms nontreated	
	Plants with foliage infection	Clean corms	Plants with foliage infection	Clean corms
	Per cent	*Per cent*	*Per cent*	*Per cent*
Check (nontreated)	28.9	73.0	88.7	0.0
4–6–50 Bordeaux mixture	0.9	84.2	16.6	34.6
20–80 Copper-lime dust	11.3	70.3	52.1	16.2

evident from the results secured that spraying, alone, will not give a satisfactory control of scab when severely diseased corms are planted, even on noninfested soil. More tests must be made before a satisfactory conclusion can be reached as to whether it will be commercially practicable to spray plants from corms that have been given an efficient treatment prior to planting.

SUMMARY

Calogreen proved by far the most efficient material tested as a disinfectant agent against the scab organism on gladiolus corms.

A 1–1000 concentration of bichloride of mercury ranked next in efficiency. A 12-hour treament with this solution gave best results. The 8-hour treatment, however, gave almost as good control.

Semesan, an organic mercury product, was fairly efficient in the control of scab, ranking somewhat below bichloride of mercury.

Formaldehyde, acetic acid, and Gold Dust were decidedly inefficient as corm disinfectants.

Calochlor, although it did reduce the amount of scab to a fair degree, delayed germination, retarded growth of the plant, and resulted in a considerable reduction in the crop of corms harvested.

There is, apparently, no difference in the response of any particular variety of gladiolus to the efficiency of the disinfectant used.

Calogreen, applied to the soil on land known to be infested, proved efficient in reducing infection from soil-borne scab organisms. Calochlor reduced such infection but delayed germination and retarded growth. Semesan gave but fair protection and lime was decidedly inefficient.

Bordeaux mixture proved notably more efficient in the prevention of foliage infection than did copper-lime dust.

DEPARTMENT OF PLANT PATHOLOGY,
MISSISSIPPI AGRICULTURAL EXPERIMENT STATION
STATE COLLEGE, MISSISSIPPI.

POWDERY MILDEW OF CRAPE MYRTLE CAUSED BY ERYSIPHE LAGERSTROEMIAE, N. SP.

ERDMAN WEST

(Accepted for publication, Dec. 1, 1932)

Powdery mildew of crape myrtle was first reported[1] in the United States in 1924 by J. J. Taubenhaus, who found it in 2 counties in Texas. Since then it has been reported[2] in six other Southern States (South Carolina, Georgia, Alabama, Louisiana, Mississippi and Florida) and the District of Columbia. The first collection in Florida was made at Gainesville in 1925 by G. F. Weber, but it has been found subsequently at widely separated points and its range in this State is probably coextensive with that of its host. Boyd reported it once on *Lagerstroemia parviflora* in Georgia but all other collections have been made on *L. indica.*

Although the disease is of variable importance from year to year, it is usually prevalent enough each season to cause a conspicuous disfiguration of crape myrtle plants. (Fig. 1, A.) This is particularly true of plants in shaded situations and in hedges. From Alabama and Georgia we have reports of instances where one hundred per cent of the leaves were affected. This is unusual, but cases where one third or more of the leaves were mildewed have been observed frequently in Florida.

The first indication of the presence of the fungus occurs in early spring before the new shoots are more than a few inches long. At this time a few abortive shoots are found projecting barely beyond the bud scales and covered with a white coating of conidia. (Fig. 1, C.) These minute deformed shoots are very inconspicuous and easily overlooked. They serve as the foci for the general infection of the plants. They also indicate, in all probability, how the fungus is able to overwinter without the interpolation of a perfect stage in its life history. Protected by the bud scales during the period that the host is dormant and leafless, the fungus is able to resume growth with the advent of spring and the appearance of succulent susceptible growth on the host.

The first conspicuous growth of the fungus occurs on the young leaves as small, circular, white patches in which the mycelial strands radiate from the center. By the time that the spot is a centimeter in diameter, conidia are being produced in the center of the spot. After a few leaves or one

[1] United States Department of Agriculture. Bur. Plant Indus. Plant Dis. Rptr. Sup. 42: 349, 1925. (Mimeographed).

[2] United States Department of Agriculture. Bur. Plant Indus. Plant Dis. Rptr. **9**: 12, 50, 1925. **10**: 22, 1926. **11**: 56, 106, 1927. **13**: 110, 1929. **15**: 103, 1931. Plant Dis. Rptr. Sup. **50**: 448, 1926. **65**: 425, 1928. **73**: 387, 1929. **75**: 71, 1930. (Mimeographed).

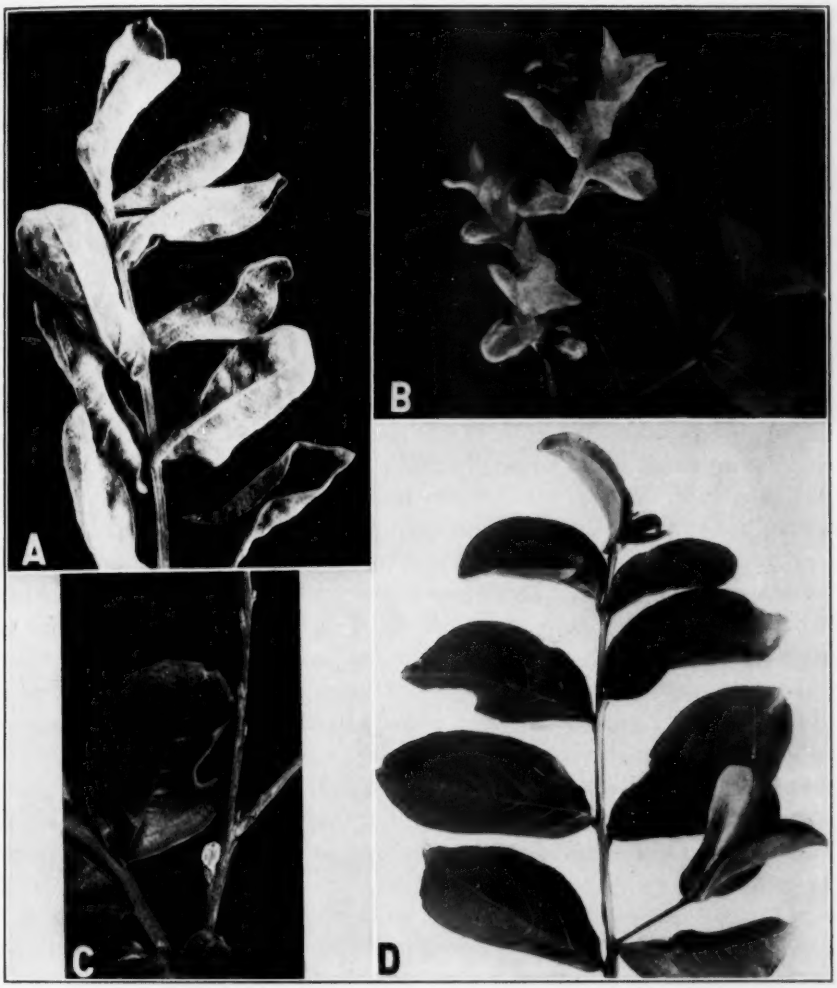

FIG. 1. A–C. Mildew on crape myrtle. A. Attacked shoot. B. Young shoot covered with spores. C. Heavily infected, aborted shoot. D. Healthy shoot.

shoot has become covered with the mildew (Fig. 1, B), the whole plant may show general infection in a week or ten days under favorable conditions. The conidia are produced in great numbers and the spread of the disease is rapid.

The disease appears first on the young shoots in early spring and continues to affect the plants visibly until the flowering spikes appear. With the advent of hot weather of midsummer, the disease apparently disappears from the plant. However, typical specimens can be found, at this time, on young sprouts near the center of the plant and on the foliage near the

ground. As fall approaches, the disease again appears on the young shoots on the outer portion of the plants and also frequently covers the older leaves as well. It is present on the plants until the leaves drop in December in Florida.

The plant parts attacked include leaves, young stems, and the various parts of the inflorescence. The affected organs soon become covered with a heavy, white, mealy coating, which is quite dusty from the innumerable spores present. When infection occurs on young organs, considerable malformation takes place. Young leaves are stunted before they attain a third of their normal size; at the same time they may become abnormally thickened. Young stems of shoots are stunted, the internodes are short and the stems irregular. When the young inflorescence is attacked, pedicels and peduncles are shortened and the buds usually fail to open. Quite frequently the diseased portions of the plant assume an unnatural reddish color beneath the white, mealy fungous growth. The diseased leaves and buds usually drop in a week or two but diseased stems frequently sprout and continue normal growth, especially when hot weather ensues.

When older leaves and stems become infected, there is little or no malformation of the organs. The leaves may even remain attached to the stem until the mealy coating weathers off, but they usually drop soon after the entire surface has been attacked by the fungus.

The organism causing crape myrtle mildew belongs to the Erysiphaceae. All reports so far published indicate that only the imperfect or oidium stage has been found in the United States. In the case of this fungus, as with other powdery mildews, the conidial stage is the conspicuous and injurious form of the organism. The description given above is that of the disease caused by this imperfect stage. However, the perfect stage of the fungus has been found once in Florida.

While the disease had been observed a number of times in Florida in 1925, no perithecia of the fungus had been observed nor had any particular effort been made to find them. Beginning with the first appearance of the mildew in the spring of 1926, the writer made frequent and careful examinations of diseased leaves, stems, and floral parts on a row of crape-myrtle bushes growing in the experimental plots of the University of Florida. As the season progressed, nothing but the conidial stage was observed. Not only were the living infected parts observed, but examinations were made of fallen and dead leaves, ripened stems and the inflorescences after flowering was past. The scanty infection during the hot weather was carefully examined, as well as the heavier infection which followed later.

Observations were continued on the brownish, mycelial coat which persisted on the older leaves and stems and early in December the ascogenous form was found. A single leaf bearing three mature perithecia was found

first and a few more before the leaves fell. In spite of exhaustive searching, no more perithecia have been found since that time, either on these particular plants or on affected plants in other localities.

In the conidial stage species of powdery mildews are not distinguishable and until perithecia are found, their exact taxonomic position must remain in doubt. The only powdery mildew bearing perithecia on crape myrtle has been *Uncinula australiana* McAlpine, reported only from Australia and Japan.[3] Therefore, previous American collections of powdery mildew on crape myrtle have been referred to this species. The perithecia of the Florida organism bear no trace of curling at the tips of the appendages, such as is characteristic of the genus Uncinula. The appendages in our species are short, erect, unmodified at the tip and distinct from the hyphae. In general, it resembles some forms of *Erysiphe polygoni* DC. in Florida, but has much smaller spores that measure only 14–17 μ long and 5–7 μ broad. Since no previously reported species has been found corresponding to these measurements and characteristics, the organism collected in Florida is therefore described as a new species.

Erysiphe lagerstroemiae, nov. sp.

Amphigenous; mycelium of two types, one evanescent and the other persistent; the evanescent type hyaline, shining and disappearing with the decline of the host, 7–9 μ in diameter; the persistent type remaining until late fall, septate, light to deep brown, rather thick-walled, frequently branched, 4–5 μ in diameter, associated with the perithecia.

Perithecia (Fig. 2, A) widely dispersed, never gregarious, globose, frequently, although not always, with a slight papilla-like knob on top, about 90 μ in diameter but varying from 80–100 μ; cells small, dark, distinct, 6–8 μ in diameter; appendages short, equalling the diameter of the perithecia in length, 90–110 μ long, 7 μ in diameter, colored yellow-brown throughout, not sharp-pointed, sometimes very slightly enlarged at point of attachment, not crowded, 6–15 in number, mostly straight or a few slightly flexuous, not interwoven with the mycelium; the perithecia attached at the base by 15–20 strands of brown, septate, flexuous hyphae of the persistent type; asci many (Fig. 2, B and C), varying from 10–23, average 16, small, ovate or broadly ovate, sometimes with a very short stalk, 30–44 μ × 17–20 μ; spores 6–8 usually 8, ovate or longer, broadest in middle, 14–17 μ × 5–7 μ; conidia borne in great numbers on evanescent mycelium, barrel-shaped, variable, hyaline, 30–34 μ × 14–17 μ.

[3] Salmon, E. S. A Monograph of the Erysiphaceae. Torr. Bot. Club Mem. **9**: 1–292. p. 118. 1900.

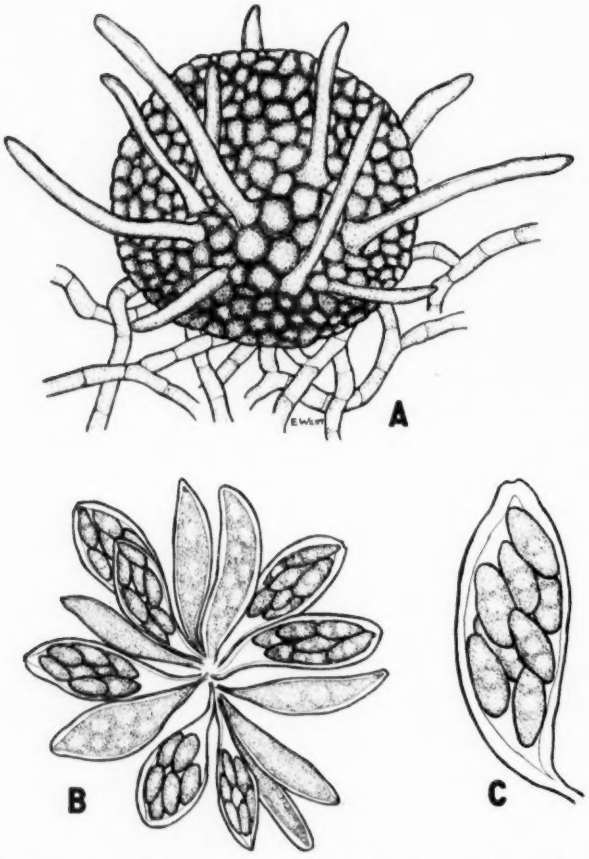

FIG. 2. *Erysiphe lagerstroemiae*. A. Perithecium showing appendages. B. Group of asci squeezed from perithecium. C. Mature ascus containing eight ascospores.

Host *Lagerstroemia indica* L.

Distribution: Texas, Louisiana, South Carolina, Georgia, Alabama, Mississippi, Florida, District of Columbia. Perfect stage reported from Florida only.

CONTROL

It has been found that a single application of commercial lime-sulphur spray diluted 1: 80 will give a satisfactory control of crape-myrtle mildew. This spray has been applied in the past whenever the powdery mildew became apparent on the foliage. Since the discovery of the overwintering stage in the buds, it is recommended that the spray be applied as soon as the buds burst in the spring, so that a general infection of the plants may be prevented.

SUMMARY

Crape-myrtle mildew, in Florida at least, is caused by a new species of fungus herein described as *Erysiphe lagerstroemiae*, nov. sp.

It is probable that this organism is responsible for the same disease in several other Southern States.

The disease is characterized by the type of injury generally associated with powdery mildews.

The perfect stage of the organism is produced only infrequently.

The fungus is capable of overwintering in the leaf buds of the host.

The disease can be controlled with commercial lime-sulphur spray.

DEPARTMENT OF PLANT PATHOLOGY,
FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

RECORDS OF CITRUS CANKER FROM HERBARIUM SPECIMENS OF THE GENUS CITRUS IN ENGLAND AND THE UNITED STATES

H. S. Fawcett and Anna E. Jenkins[1]

(Accepted for publication Nov. 14, 1932)

INTRODUCTION

For the purpose of obtaining more information on the occurrence and distribution of the disease known as citrus canker, caused by *Pseudomonas citri* Hasse (2), material of the genus Citrus (mostly cultivated) was examined in 1930 at the Herbaria of the Royal Botanic Gardens, Kew, and the Department of Botany, British Museum, South Kensington, London, England, and, in 1931, at the Gray Herbarium, and Arnold Arboretum, Harvard University, Cambridge, Massachusetts; New York Botanical Garden, New York, New York; the Philadelphia Academy of Natural Sciences, Philadelphia, Pennsylvania, and the United States National Herbarium, Washington, D. C.

Tabulations and discussions of data obtained from this study are here presented, for it is thought that they may be helpful in tracing some of the history and distribution of the disease. The history and geographical distribution of citrus canker are discussed by Tanaka (7), Peltier and Frederich (6), Fawcett and Lee (1), and others.

DATA OBTAINED FROM PHANEROGAMIC SPECIMENS

In table 1 are listed the phanerogamic specimens on which canker, or what is apparently canker, was observed. These data appear to add no new countries to the distribution of the disease.

Of the Philippine specimens 1 was collected in 1914, or the year the seriousness of the disease in the Philippines became apparent (8), although it had not yet been distinguished as canker and not scab, 4 specimens were collected the following year when the disease was actually recognized as canker (9), the others at somewhat later dates. Figure 1, A, represents one of these specimens.

The specimens of *Citrus medica* from northwestern India, possibly 1827–1831 (Fig. 1, B), and *C. aurantifolia* from Java, 1842–1844 (Fig. 1, C), however, furnish by far the earliest records yet known for the disease.

[1] Part of a paper read at the annual meeting of The American Phytopathological Society, December 30, 1931 (3). The remainder of the paper, dealing with citrus scab, has been presented separately (4). Thanks are due A. W. Hill, A. D. Cotton, W. R. Maxon, C. E. Kobuski, F. W. Pennell, J. Ramsbottom, E. M. Wakefield, and A. C. Weatherby for courtesies in connection with the examination of the phanerogamic specimens.

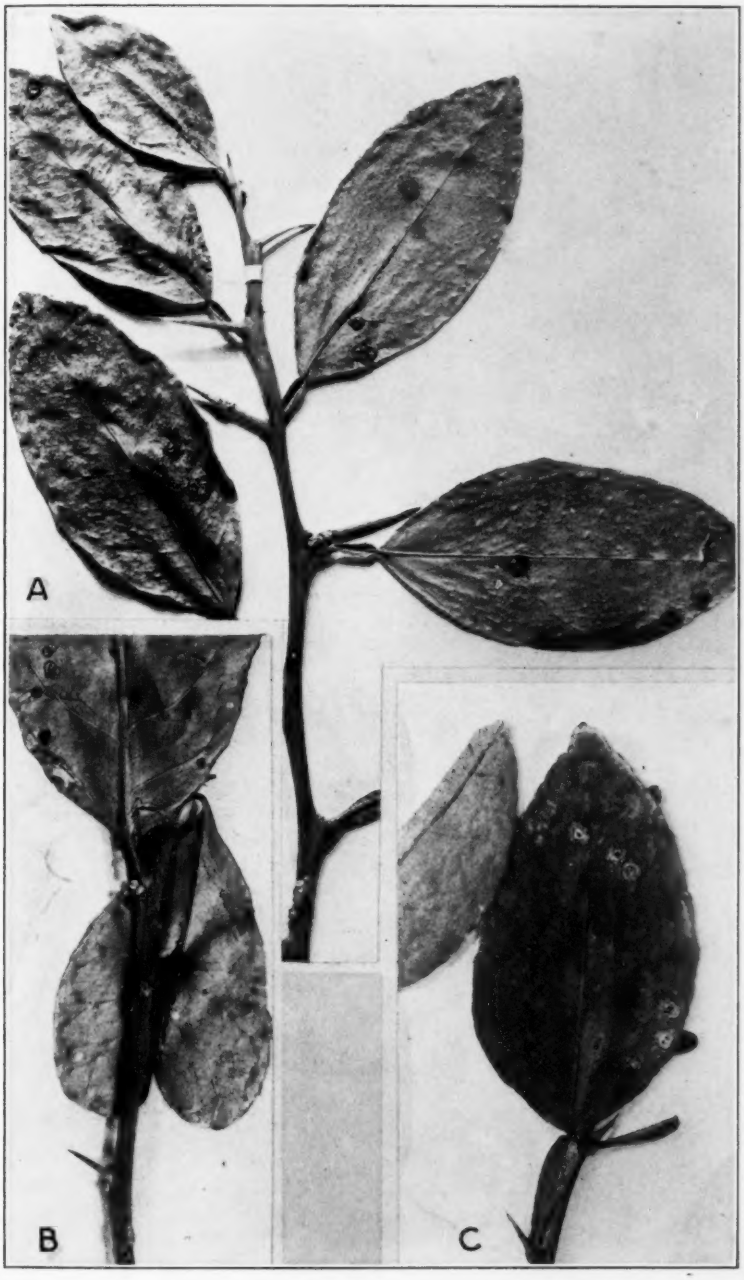

FIG. 1. Citrus canker on dried phanerogamic specimens of (A) *Citrus limetta* var. *aromatica*, Philippines, (Wester 2182); (B) *C. medica*, N. W. India; (C) *C. aurantifolia*, Java. ×1. A, photographed by M. L. F. Foubert; B and C, at Kew Herbarium.

TABLE 1.—*Identification of citrus canker or what is apparently citrus canker on phanerogamic specimens of the genus in herbaria in England in 1930 and the United States in 1931*

Herbarium	Data on specimens				Name of collection, number of specimen and collector, where given	Identification (asterisk (*) indicates that fragments of specimen showing lesions, are preserved in the Mycological Collections of the Bureau of Plant Industry)
	Date of collection	Place of collection	Kind of citrus			
			Tanaka's identification, where given	Name on label		
The Herbarium, Kew England	Early, possibly 1827–31[a]	N. W. India	*C. medica* L.	*C. medica* L.	Herbarium Royle "Herbarium Hookerianum 1867"[1]	Canker on leaves and twigs (Fig. 1, B)
"	No date on specimen, but reference to it by Moritzi (5) shows that it was collected between 1842–44	Java	*C. aurantifolia* Sw.	*C. javanica* Bl.	Plantes javanicae a cl. Zollingers lectae No. 555. In Herbarium Benthamianum, 1854	Canker on leaves. Notation on sheet shows that Tanaka also observed the numerous lesions of citrus canker on this specimen. (Fig. 1, C)
U. S. National Herbarium, U. S. A.	1903	Java	*C. hystrix* DC.	*C. hystrix* DC.	Herb. Bu. Agr. Manila No. 111, G, 5	?Canker on leaves*
"	Feb. 3, 1914	Philippines	*C. aurantifolia* (Christm.) Sw.	*C. lima* Zunan	Merrill: species blancoanae No. 436	Canker leaves severely affected*
"	March, 1915	"	"	*C. limetta* var. *aromatica* Wester	Flora of the Philippines 741–23 P. J. Wester	?Canker on leaves*

TABLE 1—(*Continued*)

U. S. National Herbarium, U. S. A.	March, 1915	Philippines	*C. aurantifolia* (Christm.) Sw.	*C. limetta* var. *aromatica* Wester	Flora of the Philippines 741–23 P. J. Wester	twigs (Fig. 1, A) Canker on leaves and
Arnold Arboretum, U. S. A.	"	"	"	*C. pseudolimonum* Wester	" 1953 P. J. Wester	Canker on leaves*
U. S. National Herbarium, U. S. A.	"	"	*C. excelsa* Wester	*C. excelsa* var. *davanenses* Wester	" 1009 P. J. Wester	Canker on leaves*
"	July, 1916	"	*C. medica* L.	*C. "limonum"* of Wester	" 116 P. J. Wester	Canker on leaves*
"	Sept., 1916	"	*C. aurantifolia* (Christm.) Sw.	*C. aurantifolia* Sw.	" (Forestry Bureau No. 26, 278)	†Canker on leaves*
"	1917	Asia	*C. depressa* Hayata	*C.* "the tachibana" *nobilis* var. *spontanea* Ito and Matsum	Arnold Arboretum Expedition to Eastern Asia 1917–18. Luikiu Islands No. 8049	Canker on leaves*
Arnold Arboretum U. S. A.	Apr. 27, 1919	Philippines	*C. hystrix* DC.	*C. micrantha* Wester	Flora of the Philippines (Forestry Bureau No. 27, 436)	Canker on leaves.* Lesions on same specimen as presented in U. S. National Herbarium are apparently those of canker.*

[a] Date of possible collection by Royle as suggested in letter from Dr. R. N. Parker, Forest Research Institute, Dehra Dun, U. P. India For further discussion see text.

Notations on the Javanese specimen indicate that the canker lesions on this specimen were observed by Tanaka, presumably in 1928, when he identified it as *C. aurantifolia.* In correspondence regarding the specimen from the Royle Herbarium, collected in northwest India, contained in the Herbarium Hookeriarum, Dr. R. N. Parker, Forest Botanist at the Forest Research Institute, Dehra Dun, has recently suggested that this may be Royle 7. He adds, "It is probable that Royle collected his specimen of *Citrus medica* in one of the ravines near Dehra Dun where the plant is not uncommon." This, if correct, means that the specimen was collected in 1827–1831. These data indicate the probability that citrus canker originated in India and Java rather than in other regions of the Orient.

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CONTROL OF OAT SMUT[1]

V. H. Young and C. K. McClelland

(Accepted for publication Oct. 26, 1932)

The control of the smuts of oats by the use of formaldehyde solutions applied in a variety of ways is well established. When properly carried out, treatment with formaldehyde has given excellent control of both the loose and covered smut of oats. Recently, several workers have reported efficient control of these smuts by the use of organic-mercury dusts and by the use of formaldehyde adsorbed on some inert carrier. Karns[2] also has reported that elemental iodine, dissolved in carbon bisulphide, is efficient for this purpose.

Experimental tests in 1931 and 1932, at the University Farm, Fayetteville, Arkansas, of some of these newer oat-smut treatments gave rather striking results, which are here reported. Seed of Fulghum oats obtained in 1930 from a badly smutted plot on the university farm was used in these experiments in 1931 without the addition of further inoculum. Seed from the nontreated checks of 1931 was used in 1932. The following dusts were applied in 1931: Ceresan, active principle 2 per cent ethyl mercury chloride; Dubay No. 952 K, active principle ethyl mercury tartrate 2 per cent; Dubay No. 952 C, active principle ethyl mercury tartrate 1 per cent; Dubay No. 500 LL, active principle ethyl mercury arsenate 1 per cent; Merko, the active ingredient of which is said to be an inorganic mercury compound; Corona Oat Dust, said to be activated formaldehyde; and Corona No. 174 E, said to contain a soluble organic mercury compound. In addition to the dust treatments, seed for one plot in 1931 and for 3 plots in 1932 was treated with a 10 per cent solution of iodine in carbon bisulphide at the rate of ½ oz. of elemental iodine per bushel. In 1932 Dubay No. 1134, active principle ethyl mercury phosphate 2.5 per cent, and the liquid-formaldehyde treatment[3] were added to the tests. Merko, which failed to control oat smut in 1931, and Corona No. 174 E, which was less effective than the other dusts employed in 1931, were discarded.

Dusts were applied by means of a barrel churn to which a baffle plate had been added at one end to secure more thorough mixing. Each batch

[1] Contribution from the Departments of Plant Pathology and Agronomy. Published with the approval of the Director of the Arkansas Agricultural Experiment Station as No. 322, of the University of Arkansas Journal Series.

[2] Karns, George M. Effectiveness of iodine in the control of smut on oats. Ind. & Eng. Chem. 22: 864. 1930.

[3] Formaldehyde was applied by the spray method as described by Tapke, V. F. Formaldehyde seed treatment for oat smuts. U. S. Dept. Agr. Misc. Pub. No. 21. 1928.

of seed was rotated for 5 minutes and the interior of the churn was washed out and dried between treatments. Dusts were applied at the rate of 3 oz. per bu. except Dubay No. 1134, which was employed at the rate of 1 oz. per bu., according to the recommendation of the manufacturers. The iodine in carbon bisulphide solution was added to the oats in a glass fruit jar and the oats were then shaken until uniformly discolored by the solution. They were then spread out in a thin layer to allow evaporation of the carbon bisulphide.

Treated oats were planted in 1931 in $\frac{1}{10}$-acre plots on land that had been in corn for the 3 preceding years, each treatment being repeated 3 times with the exception of the iodine in carbon bisulphide treatment, of which there was only 1 plot. Seven nontreated checks were used, the last in the first and second series appearing as the first in the series following. All seed for the 1931 tests was sown March 18, 1931, 2 days after treating. Soil moisture and temperature conditions remained favorable for the growth of oats throughout their growing period. Stand counts and counts of infected heads were made shortly before harvest, when all heads had appeared. By means of a wire hoop, 22 in. in diameter, 10 samples were taken at random from each plot. In certain cases, though these counts indicated that no smut was present, a careful survey of the plots revealed the presence of a very small number of smutted panicles. These were equivalent to a mere fraction of one per cent of the total number of panicles in the plot. A thorough search of all plots indicated that loose smut (*Ustilago avenae*) was the predominating form, both in 1931 and 1932.

The dust treatments used, the percentage of infection in each plot, the average percentage of infection for each treatment, the threshing percentage, yields in bushels per acre, and the gains or losses in bushels per acre are given in table 1. Theoretical yields of interpolated checks have been used as a basis for computing gain or loss from treatments over checks, and gains or losses without adjustment also are shown with percentage values.

A statistical interpretation of the yield data given in table 1 shows that the standard deviation of an individual plot yield as determined by the check-plot method is 11.66 bu. or 31.3 per cent. The standard deviation of the mean for 3 plots is 6.74 bu. The difference between plots necessary for significance is $2\sqrt{2}$ 6.74 or 19 bu., assuming that the treated plots are subject to the same error in bushels as the nontreated ones. According to this standard, no significant differences in yield exist, due to the high variability as shown by the check plots. However, the reduction in the incidence of smut resulting from all treatments except Merko, which the manufacturers do not recommend for oats, is clear-cut and, on the whole, satisfactory. As shown in table 1, there seems to be little relation between the treatments

TABLE 1.—*Effect of dust treatments on the incidence of smut on Fulghum oats and on yield at University Farm, Fayetteville, Arkansas, 1931*

Treatment / Series	Percentage of infection from counts of 10 areas 22″ in diameter				Threshing percentage	Yields per acre				Gains		
	A	B	C	Averages		A Bu.	B Bu.	C Bu.	Aver. yield Bu.	Bu.	Bu.[a]	Per cent
Check	15.92	20.46	20.11	20.08	50.67	59.2	34.8	26.3	37.3			
	19.96	22.24	23.51			42.7	24.8	35.9				
	20.46	20.11	18.36			34.8	26.3	50.6				
Dubay No. 952 K	.09	0	0	.03	57.02	53.9	35.6	28.1	39.9	0.94	2.6	7.0
Ceresan	0	0	0	0	55.54	50.9	37.8	34.7	41.2	3.31	3.9	10.5
Dubay No. 500 LL	0	.96	.52	.49	54.54	48.0	40.5	38.8	42.4	5.68	5.1	13.7
Corona No. 174 E.	3.02	2.37	2.91	2.77	56.48	52.7	35.5	40.6	42.9	7.32	5.6	15.1
Merko	18.94	20.32	19.89	19.72	54.60	37.3	36.7	34.4	36.2	0.98	−1.1	−3.0
Dubay No. 952 C	0	0	0	0	53.37	44.1	41.7	55.3	47.0	11.17	9.7	26.1
Corona Oat Dust	0	0	0	0	55.04	40.9	38.9	55.3	45.1	8.5	7.8	21.0

[a] These gains obtained without adjustment.

and the ratio of grain to straw as expressed by the threshing percentage,[4] and no relation between treatments and stand could be noted.

The 1932 tests were planted March 19, 1932. Unusually dry weather during the spring months resulted in short straw and a low yield of grain. The dust applications were made as in the previous year and 3 plots from each lot of treated seed were again planted. All seed was sown as soon as treated, with the exception of that treated with formaldehyde and that treated with iodine, which was allowed to stand 24 hours before planting. Five check plots were included. As mentioned previously, the seed used in 1932 was obtained from one of the check plots of the 1931 tests in which about 20 per cent of the panicles were infected. Five areas on each plot and then 5 duplicate areas on the 5 check plots were counted and percentages of infection calculated.

Table 2 gives the results of the counts as percentage of infection and also the yields of the various plots in bushels per acre.

As will be seen from table 2, the percentage of infection on plots where nontreated seed was used was 19.25 per cent, which was not markedly different from that of the previous year. All of the treatments gave good control of smut except the iodine treatment, which was not nearly so effective as in 1931, possibly because thorough coverage was not secured. The dust treatments were all effective, several of them showing perfect control.

On the plots having treated seed there was a consistent, though not large, gain in yield, varying from 2.7 to 6.8 bu., or 12.9 to 32.5 per cent. These figures are from nonadjusted yields; no interpolations between check plots were made because, due to amount of seed available, the plots varied in size. The gains averaged 4.3 bu. or 20.55 per cent on the plots where dust-treated seed was used in contrast to a gain of only 17.7 per cent for the liquid-formaldehyde treatment.

A statistical treatment of the yield data shows that the standard deviation of a single yield was 2.16 bu. or 10.3 per cent, showing the variability to be only one-third as much as in 1931. The standard deviation of three plots is 1.25 bu. Again using twice the square root of 2 multiplied by the standard deviation of 3 plots as a measure of significance, we have 3.5 bu., the magnitude of a difference needed to establish significance. All treatments except Dubay 1134 and iodine may be taken as having given significant differences in yield. The increased yields of certain of the dust treatments over the liquid formaldehyde treatment suggest the possibility of stimulatory effects due to the treatment in addition to increases from the control of oat smut, such as have been noted recently by workers in other

[4] The threshing percentage represents the relation between the weight of threshed grain and the weight of bundles.

TABLE 2.—*Effect of dust treatments, liquid formaldehyde, and iodine in carbon bisulphide on the incidence of smut on Fulghum oats and on yield at University Farm, Fayetteville, Arkansas, 1932*

Treatment / Series	Percentage of infection from counts of 5 areas 22″ in diameter						Yield per acre				Gains	
	A	B	C			Averages	A Bu.	B Bu.	C Bu.	Aver. yield in bu.	Bu.	Per cent
No treatment	21.6	18.2	27.9	20.	14.7		23.5	23.3	20.1			
dupl. 5 counts	17.3	20.8	17.5	20.5	14.	19.25	18.6	18.8		20.9		
Dubay No. 1134	0	0	.4			.13	27.8	21.0	22.2	23.7	2.8	13.4
Dubay No. 500 LL	0	1.3	.4			.57	24.8	24.8	23.5	24.4	3.5	16.7
Dubay No. 952 K	0	0	0			0	25.5	23.1	23.7	24.1	3.2	15.3
Dubay No. 952 C	0	0	0			0	28.	26.1	29.1	27.7	6.8	32.5
Ceresan	0	0	0			0	28.5	23.5	25.1	25.7	4.8	23.
Corona Oat Dust	0	0	0			0	25.5	22.3	29.0	25.6	4.7	22.5
Formaldehyde	0	.9	0			.3	24.0	23.3	26.4	24.6	3.7	17.7
Iodine	5.	4.8	5.2			4.65	21.2	24.8	24.9	23.6	2.7	12.9
dupl.		3.6										

States. Sufficient data, however, are not available to draw definite conclusions.

The results of the iodine-carbon bisulphide treatment of oats are not included in table 1, as only one plot was used in 1931. Counts of 10 areas selected at random, as in the case of the other plots, gave no smut whatsoever in the iodine plot, and even a careful search of the whole plot revealed the presence of only the slightest trace of smut. The yield per acre computed from the iodine plot was exceptionally high, but it is believed that soil differences may be responsible for part of this increase. In 1932 iodine gave much poorer control than did the other treatments, and the increase in yield, when tested statistically, did not appear significant.

SUMMARY

Certain dust treatments, employed for 2 years, gave excellent control of loose smut of oats, whereas others gave only a fair degree of control or were ineffective. In 1932 the dust treatments compared favorably with the liquid-formaldehyde treatment.

Iodine, dissolved in 10 per cent solution in carbon bisulphide, applied at the rate of ½ oz. per bu., gave nearly perfect control of loose smut of oats in 1931 but was much less effective in 1932.

None of the treatments employed appeared to affect the stand or to have any marked effect on the ratio of straw to grain as expressed in the threshing percentage.

Variability in the check plot yields in 1931 was so great that significant statistical differences were not made. In 1932 less variability was shown and the gains, for the most part, attained significance.

ARKANSAS AGRICULTURAL EXPERIMENT STATION,
FAYETTEVILLE, ARKANSAS.

ROGUING AS A MEANS OF CONTROL OF TOBACCO MOSAIC

FREDERICK A. WOLF[1]

(Accepted for publication Nov. 3, 1932)

It has long been known that mosaic-infected plants in the field serve as sources of inoculum for near-by healthy plants. As long ago as 1904, Selby[2] suggested the prompt removal, from fields, of diseased plants when they were noted early in the growing season. More recently Valleau and Johnson[3] regard this practice as advisable. Apparently, however, no experimental data are available on the effectiveness of this procedure. Accordingly, the writer undertook to test the efficiency of roguing as a field practice in mosaic control.

The fields used for these trials were set with healthy plants, as determined by successive examinations of the seed beds. These examinations were made at intervals, beginning prior to transplanting and extending through the transplanting season. The selected fields had borne a mosaic-affected crop during the previous year, and their soils were, in consequence, infested. The writer's observations indicate that, even though fields are set with mosaic-free seedlings, mosaic invariably develops, provided such fields have produced a diseased crop during the preceding season. Infested soil, in such cases, constitutes the initial source of inoculum. Moreover, these observations show that when infested fields are examined as soon as the plants have become established, following transplanting, they will be found to contain an average of less than one per cent of affected plants. These plants then serve as sources for the increase of the disease.

The removal of mosaic plants, as early as possible, was the procedure employed in the present trials with the results summarized in the following tabulation.

It may be noted, first of all, that roguing did not result in eradicating mosaic except in fields 1 and 8. Among a total of 48,013 plants in these 13 fields, 177 diseased ones were destroyed, and at the beginning of harvest 162 mosaic ones remained. When the first examination was made some of the inoculated plants had been transplanted for too short a period to allow sufficient time for the development of symptoms. Such plants were plainly mosaic at the date of second inspection. The removal of diseased plants at these 2 inspections should have resulted in the complete eradication of

[1] Pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] SELBY, A. D. Tobacco diseases and tobacco breeding. Ohio Agr. Exp. Sta. Bul. 156: 87–114. 1904.

[3] VALLEAU, W. D., and E. M. JOHNSON. Observations and experiments on the control of true tobacco mosaic. Ky. Agr. Exp. Sta. Bul. 280. 1927.

TABLE 1.—*Results of roguing of mosaic plants as a means of control*

Year	Field No.	Number of plants rogued	Date of removal from field	Total number of plants removed	Number of plants remaining at maturity of crop	Total number of plants in the field
1928	1	2	June 18	2	0	4,050
1929	2	18 2	June 8 " 21	20	3	2,813
"	3	11 5	June 25 July 5	16	7	1,560
"	4	5 15	June 25 July 1	20	9	2,585
"	5	11 3	June 19 July 1	14	3	3,440
"	6	2 8	June 19 July 1	10	8	1,095
"	7	6 12	June 17 " 29	18	10	4,080
"	8	1 1 2	June 17 " 29 July 9	4	0	5,600
"	9	11 4	June 25 July 5	15	7	1,560
1930	10	10 4	June 6 " 18	14	52	3,050
"	11	3 2	June 5 " 19	5	5	8,520
"	12	2 9	June 21 July 1	11	5	2,160
"	13	10 6 12	May 28 June 5 " 19	28	53	7,500
Total				177	162	48,013

mosaic from all fields. By the time that the crop was ready to harvest, however, the growers had introduced mosaic anew from near-by affected crops, into all except fields 1 and 8. This explanation is supported by the fact that the tops and suckers, instead of the entire plants, were mosaic, and that the crops in adjoining fields were diseased.

It would have been interesting to have known what proportion of the total number of plants in these 13 fields would have become mosaic by

harvest time if roguing had not been employed. Since it was impossible to do this or to have entirely satisfactory check experiments in adjoining parts of the same fields, comparisons must be made in another way. Abundant data are at hand for such comparisons, since records have been made, over a 3-year period, of the number of mosaic plants in 125 fields in which infested soil constituted the source of inoculum. The average initial infection in 12 representative fields of this type was 0.7 per cent, and by harvest time an average of 13.5 per cent had been diseased. With this as a basis for comparison, 336 plants in the 13 fields should have been mosaic at first, and by harvest time this number should have increased to 6,481. The actual presence of 339 plants, approximately half of which were a total loss because the plants were removed, instead of an expected 6,481, among a population of 48,013, constitutes very satisfactory control.

No effort was made to replant with mosaic-free seedlings in these 13 fields to replace the 177 plants that were removed. The resetting of healthy plants in place of mosaic ones cannot be recommended as a general practice, since the experience of all who have attempted to do this shows that a considerable proportion become inoculated by being set where diseased ones have been removed. This is supported by the results in the following case in which approximately 98 per cent of the plants in a particular field were found to be infected when the crop was cultivated for the first time. Accordingly, all of the plants from a portion of this field were removed and the area was reset with healthy plants. Twenty-seven days later 16 per cent were mosaic, 13 days thereafter the number had increased to 25.5 per cent, and after an additional 11 days 57.1 per cent were affected.

Attention should also be called to the fact that the roguing of crops set with plants from infested seed beds was not attempted. In such cases, experience has shown that the initial infection is too great to permit such a procedure to be profitable since upwards of 10 per cent of the plants are visibly diseased as soon as they have become established in the field.

SUMMARY

When healthy plants are set in fields which produced a mosaic crop during the preceding season, normally only a small proportion of the plants are infected at time of first cultivation. Under these conditions, roguing early in the season is an effective check on the spread of mosaic.

Rouging of fields set with seedlings from infested seed beds is not advisable, because it necessitates the removal of too large a proportion.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

EFFECT OF MOSAIC OF FLUE-CURED TOBACCO ON YIELD AND QUALITY

FREDERICK A. WOLF[1] AND E. G. MOSS[2]

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Most growers of flue-cured tobacco are of the opinion that mosaic causes losses through decrease in yield and impairment of quality. Some maintain, however, that it does no damage. Within the past few years the results of several investigations on this phase of the mosaic problem with other types of tobacco have been published. Valleau and Johnson[3] found that mosaic reduced the yield of Burley tobacco approximately one-third and the value two-fifths when all plants were inoculated at time of transplanting. When inoculation was delayed until topping time, there was no decrease in yield, but there was a decrease of one-fourth in crop value. With Maryland tobacco, McMurtrey[4] found that the average reduction in yield for a 3-year period was 30 to 35 per cent, and in value, 55 per cent when all plants were inoculated at time of transplanting. The damage was almost as great as this when the plants were inoculated a month after transplanting time. There was no significant reduction in yield, however, when inoculation was delayed until time for topping; yet, even at this stage, mosaic adversely affected the quality.

Since there are no available data on the effect of mosaic on yield and quality of flue-cured tobacco, such studies were undertaken in 1929 and 1930 at the Tobacco Experiment Station, Oxford, N. C. Four adjacent plots of $\frac{3}{8}$ acre each were set with tobacco. All were planted on the same day, were given equal applications of fertilizer, and cultivated alike. The first was inoculated when the plants were set, the second a month later, the third at time of topping, and the fourth was left uninoculated as a basis for comparison. The inoculum was prepared by crushing with the fingers the leaves of a green mosaic plant and suspending the macerated material in a small quantity of water. The tops of the seedlings were dipped into this inoculum just prior to transplanting, in the case of those that were inoculated when they were transplanted. The inoculum, simi-

[1] Pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] Agronomist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture and Assistant Director, Tobacco Branch Station, North Carolina Department of Agriculture.

[3] Valleau, W. D., and E. M. Johnson. The effect of a strain of tobacco mosaic on the yield and quality of Burley tobacco. Phytopath. 17: 523–527. 1927.

[4] McMurtrey, J. E., Jr. Effect of mosaic disease on yield and quality of tobacco. Jour. Agr. Res. 38: 257–267. 1929.

larly prepared, was communicated to the block of plants inoculated a month later, and to those inoculated at topping time by wetting the fingers with inoculum, and then gently rubbing, between the thumb and fingers, the bud leaves. Nearly all plants inoculated developed evident symptoms within 2 weeks. Those inoculated at time of transplanting remained stunted during the first portion of the growing season, but were normal in size by the end of the season. Moreover, they matured about 2 weeks later than the plants on the noninoculated plot.

The results of these tests for the season 1929 and 1930 are presented in the following table.

TABLE 1.—*Influence of mosaic on yield and quality of flue-cured tobacco*

Plot No.	Year	Time of inoculation	Yield per acre	Decrease in yield	Acre value	Decrease in acre value
			Pounds	*Per cent*		*Per cent*
1	1929	At transplanting	668	23.2	166.22	42.3
	1930	do	642	39.6	29.24	66.8
Average			655.0	31.4		54.6
2	1929	One month after transplanting	646	25.7	153.42	47.2
	1930	do	739	34.6	57.46	37.0
Average			692.5	30.1		42.1
3	1929	At topping	734	15.6	208.00	23.6
	1930	do	917	18.8	79.37	23.9
Average			825.5	17.2		23.8
4	1929	Control, not mosaic	870		272.59	
	1930	do	1,130		91.24	
Average			1,000			

It is evident that mosaic adversely affects both the yield and quality of flue-cured tobacco, dependent upon the stage of growth at which infection occurs. The 2-year average yield on the mosaic-free plot was 1,000 pounds; that on the plot inoculated at time of setting the plants was 655 pounds, or a decrease in yield of 31.4 per cent and a decrease in value of 54.6 per cent. The yield on the plot inoculated approximately a month after transplanting was 692 pounds, a reduction in yield of 30.1 per cent and in value of 42.1 per cent. The plot inoculated at time of topping yielded 825 pounds, a decrease in yield of 17.2 per cent and in value of 23.8 per cent.

Only in rare cases, when judged by the writers' observations, which have extended over three seasons, is mosaic, in any grower's crop, so destructive as in plots 1 and 2. However, an average of approximately 20 per cent of the total crop may be mosaic at time of topping. The loss occasioned by this proportion, plus that resulting from the wholesale inoculation that occurs during the operation of topping, should result in a decrease in yield and value in excess of that shown in plot 3. At any rate, these data show the necessity for preventing infection and for the employment of cultural practices that will reduce the spread of the disease.

SUMMARY

Mosaic seriously reduces the yield and impairs the market value of flue-cured tobacco. The degree of damage is related to stage of growth at which infection occurs, for, when infection occurs early in the growth of the crop, it occasions the greater losses.

PHYTOPATHOLOGICAL NOTES

Downy Mildew of Tobacco on Pepper, Tomato, and Eggplant.—Downy mildew of tobacco (*Peronospora hyoscyami*, de By.) was first widely prevalent in the tobacco area of eastern South Carolina in 1932. Soon after the tobacco plants in the beds became diseased, reports were received that pepper plants in beds also were diseased. The growers were convinced that the disease of pepper was caused by the downy mildew or blue-mold fungus because of the similarity of effects on the plants. This belief was strengthened by the fact that in the 30 years of culture of the Cayenne type of peppers in the vicinity of Florence, South Carolina, no serious difficulty had been encountered in growing the plants in beds prior to the appearance of the tobacco disease.

A Peronospora was found on some pepper plants of one lot sent to the senior writer, while it was not found on the plants of another shipment. Plants later collected by the writers showed the same fungus but it was not abundant. Young pepper plants were killed when attacked by the fungus in 1932. Some plant beds were seen with nearly every plant destroyed.

The first diseased pepper plants were noted on April 21, 1933, in a row at the end of an infected tobacco-plant bed, and diseased plants in several pepper beds were observed within the following 3 days. A Peronospora was found on about one-third of the diseased leaves. If infection occurred when the plants were almost large enough to be set in the field, as was generally the case in 1933, the infected leaves were shed, but few plants were killed in contrast to the heavy mortality of young plants in 1932. This article may be of interest at this time because of the recent reports[1] that a fungus apparently identical with *Peronospora hyoscyami* has been found on pepper and also on tomato.

On April 24, 1933, 2 sq. yd. of a pepper-plant bed were used in a cross inoculation of *Peronospora hyoscyami* from tobacco to pepper. An adjoining noninoculated area of the same size was used as a check. The beds were watered and the plants then covered with a mist of water from a small hand sprayer. Tobacco leaves showing a dense coating of fresh spores were shaken above and then dragged over the plants in one bed. A muslin cloth was then placed over the inoculated and noninoculated beds and moistened at intervals of approximately 1½ hours during the daylight hours of the 2 following days. Three days following inoculation, a few diseased plants were seen scattered throughout the large bed, a part of which was being used for the experiment. Infection conditions were thence unfavorable and,

[1] Plant Disease Reporter **17**: 37, 1933.

fortunately, there was no further spread of natural infection. At the end of 7½ days, 85 per cent of the plants in the inoculated area showed definite symptoms of the disease, while only about one per cent of those in the check area showed the disease.

Eight sweet pepper plants of an unknown variety growing in pots were inoculated from infected tobacco plants on May 6, 1933. Four pots of plants used as checks were not inoculated. Definite symptoms of disease were evident on the plants in 3 pots of the inoculated group 5½ days later and a plant in a fourth pot was found diseased on the eighth day after the inoculation was made. The noninoculated plants remained healthy.

An attempt was made to infect tobacco plants with the fungus from pepper; within 42 hours after inoculation, practically all of the plants in both the inoculated and noninoculated areas were showing an abundance of Peronospora spores and sporophores on the under surface of the leaves. This was accepted as rather conclusive evidence that natural infections had occurred before the inoculations were made. No further inoculations from pepper to tobacco could be made, since all tobacco plants at the Experiment Station became diseased a few days after the first inoculation.

A Peronospora apparently identical with that on tobacco has been found by the writers on tomatoes as reported by others[2] and also on a new host,

TABLE 1.—*Frequency distribution of spore size of a Peronospora on tobacco, tomato, pepper, and eggplant*[a]

Classes μ	Length				Width			
	Tobacco	Tomato	Pepper	Eggplant	Tobacco	Tomato	Pepper	Eggplant
8.1							1	1
10.8–12.2	2		1		21	51	15	234
12.4–13.9	2	3	2	8	127	207	223	278
14.4–15.1	5	5	9	27	116	147	149	60
15.3–16.3	81	128	60	194	266	182	194	25
17.2–18.2	103	109	75	127	43	8	13	0
18.6–19.8	180	197	209	168	22	5	5	1
20.1–21.1	94	74	84	42	4			1
21.7–22.9	98	74	124	26	1			
23.0–24.4	25	9	34	7				
24.9–25.7	3	1	2	1				
27.1	7							
Total	600	600	600	600	600	600	600	600

[a] Average size of spores on tobacco 19.2 × 15.4 μ; tomato 18.6 × 14.6 μ; pepper 19.5 × 14.9 μ; eggplant 17.9 × 13.1 μ.

[2] Plant Disease Reporter. See footnote 1.

the eggplant. The tomatoes and eggplants were growing in a bed a few feet away from tobacco beds.

Measurements of 600 spores each from tobacco, pepper, tomato, and eggplant have been made and the results are presented in table 1. Similarity in spore size is evident.

The appearance of a distinctly new disease of pepper, tomato, and eggplant since the recent introduction of *Peronospora hyoscyami* on tobacco, the close association of the diseased plants with infected tobacco plants, and the apparent morphological similarity of sporophores and spores, seem to indicate that *P. hyoscyami* can attack these plants. The foregoing facts together with the successful infection of pepper plants on two occasions with spores from tobacco seem to be fairly certain evidence that *P. hyoscyami* will infect pepper.—G. M. ARMSTRONG AND W. B. ALBERT, South Carolina Experiment Station, Clemson College, South Carolina.

Manufactured Tobacco, a Source of Inoculum for Mosaic in Flue-cured Tobacco.[1]—Consideration has been given, since the spring of 1928, to the source of mosaic virus in seed beds in North Carolina. Observations on the time of first appearance of tobacco mosaic in the beds, on its peculiar distribution therein, and on the presence of visible symptoms on the youngest leaves of seedlings whose older leaves are apparently normal lead one to conclude that in many cases the virus is introduced by the grower through the use of various forms of manufactured tobacco. Manifestly, such epiphytological evidence does not constitute unequivocal proof. The following studies were made, therefore, for the purpose of determining whether, by direct experimentation, it could be found that manufactured tobacco is a source of mosaic virus, as has been reported by Valleau and Johnson[2] and by Fukushi.[3] Since manufactured tobacco is apparently the most important source of inoculum for mosaic in seed beds in flue-cured tobacco districts, a fact not generally known, the publication of the present report, which confirms the findings of other investigators and is supported by a considerable body of unpublished epiphytological data, seems to be warranted.

Two lines of evidence were followed in testing the infectiousness of tobacco. The first involved the use of air-cured mosaic-affected leaves, and the second of different forms of manufactured tobacco procured from retail stores.

[1] This report, prepared by the junior writer, is based upon studies by Hans J. Busch, Berlin-Spandau, Germany, during his residence as a graduate student at Duke University.

[2] VALLEAU, W. D., and E. M. JOHNSON. Commercial tobaccos and cured leaf as a source of mosaic disease in tobacco. Phytopath. 17: 513–522. 1927.

[3] FUKUSHI, T. On the modes of transmission of the mosaic disease of tobacco. Jour. Sapporo Soc. Agr. & For. 22: 305–320. 1931.

Mosaic-affected leaves were collected in August, 1928, were then air-cured, stored in cans, and through the courtesy of a manufacturer, were converted into chewing tobacco during April, 1929. This tobacco was employed in the spring and summer of 1929 in making a series of inoculations. It was applied to healthy plants either as spittle or was sprinkled upon them. In the latter case the inoculum was prepared by soaking a small quantity of tobacco in water. Rapidly growing potted plants, when inoculated, contracted mosaic within a period of about 2 weeks. Although all tobacco grown in the Coastal Plain and Piedmont sections of North Carolina is flue-cured, and the temperature reached during the last stages of curing usually destroys the virus, yet the infective agent occasionally survives the curing process. Even if it were destroyed in all flue-cured tobacco, manufactured tobacco might still remain a source of inoculum, because all brands of granulated, plug, and cigarette tobaccos are blended and, consequently contain air-cured tobacco grown in sections where mosaic is annually a more or less generally prevalent disease.

The studies bearing on the infectiousness of 45 brands of manufactured tobacco involved an examination of 11 brands of snuff, 17 of chewing tobacco, 10 of granulated pipe tobacco, and 7 of cigarettes. Duplicate samples of 15, and triplicate samples of 4 of the brands were tested, making a total of 68 samples. The samples were placed in flasks of sterilized water, and there allowed to soak for about 12 hours. This material was then used as an inoculum. Sterilized cotton swabs on sticks were dipped into the inoculum and the latter was gently brushed upon the buds of healthy plants. Three plants were inoculated with each sample. Sterile swabs were thus employed with each sample. This method has the additional advantage of preventing accidental inoculations, since the plants were not touched directly by the operator. After 12 to 18 days some of the plants inoculated with 2 of the brands of chewing tobacco and 2 of snuff showed symptoms of mosaic, while those inoculated with the remaining 41 brands remained mosaic free.

Infectiousness was verified in each case by testing another sample from the same package. In some cases only one of the 3 inoculated plants became mosaic. A similar failure of all inoculated plants to develop symptoms was reported by Valleau and Johnson.[4] In some cases one sample of a particular brand contained the virus, while another gave no infection. At least it may be concluded from these experiments that occasional samples of certain brands of manufactured tobacco contain the mosaic virus. These results support those of Valleau and Johnson,[4] who found among manufactured tobaccos 4 brands of plug, 7 of cigarettes, and 5 of granulated

[4] *l. c.*

tobacco that were infectious. They also support the more recent results of Fukushi[5] who reported that 25 among 30 packages of 4 brands of pipe tobacco were infectious. He inoculated plants by rubbing the pipe tobacco between the fingers and then, with contaminated fingers, slightly crushing the leaves.

It may be noted that in the present studies cigarettes and granulated smoking tobaccos were not found to be infectious. Valleau and Johnson's[6] studies led them to conclude that these forms of tobaccos are of minor importance in the mosaic problem.

When consideration is given to the readiness with which virus is spread, it is apparent that the presence of mosaic in the seed beds is an exceedingly important matter, even though only a few plants are affected. This fact is scarcely appreciated except by those who have had experience with tobacco mosaic.—HANS J. BUSCH AND FREDERICK A. WOLF.

Correction in Authority for Cronartium ribicola.—As pointed out by Sydow,[1] and independently confirmed by the writer, *Uredo ribicola* Lasch does not belong in the genus Cronartium, as heretofore assumed by the writers[2] and others. This fact requires the correct citation to be *Cronartium ribicola* Fischer, according to taxonomic procedure under the International Rules of Nomenclature. A full statement of the situation will probably be made by Sydow in the Annales Mycologici.—J. C. ARTHUR, Lafayette, Indiana.

[5] *l. c.*

[6] *l. c.*

[1] SYDOW, P. & H. Monographia Uredinearum 4: 590. 1924.

[2] ARTHUR, J. C. Phytopathology 23: 559. 1933.

ANNOUNCEMENT

The session at Boston, December 28 to December 30, 1933, will be the 25th annual meeting of The American Phytopathological Society. In recognition of this event it is hoped that the president, Doctor Arthur, dean of American mycologists and phytopathologists, will speak briefly on progress in Phytopathology during this period and on some of the great personalities in this field.

The Society will hold joint sessions with: (1) Section G and other Botanical Societies, Thursday afternoon; (2) the Mycological Society, Friday; and (3) the Potato Association of America, Saturday. On Thursday afternoon the annual conference on extension work in plant pathology will be held.

Our headquarters hotel, the Westminster, has some very attractive suites for 3 to 6 persons. To assist the managers in arranging accommodations for the December meeting, it is suggested that members write the hotel at once concerning reservations, informing them of instances where groups would prefer to be located together or in near-by rooms, or of desire on the part of two individuals to share a double room.

Scientific sessions will be held in the Biological Laboratories, Harvard University, where there will be space for exhibits of slides, charts, and other material related to papers presented. Packages containing material for exhibition should be marked Phytopathological Exhibit and be mailed or expressed to Dr. W. H. Weston, Jr., Biological Laboratories, Divinity Avenue, Cambridge, Massachusetts.

Business meetings will be held as usual on the mornings of Thursday and Saturday.

The annual dinner of the Society will be Thursday evening. While it is not possible at this time to announce either the participants or their subjects, it seems certain that the program for this classic event will be in line with the best traditions of the Society.